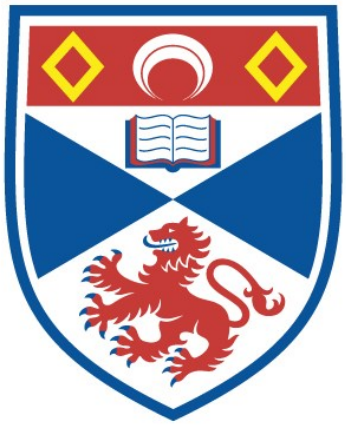
THE ABSORPTION OF AMINO ACIDS FROM THE MAMMALIAN INTESTINE

John Basil Wilson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1949

A THESIS

PRESENTED FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

OF

THE UNIVERSITY OF ST ANDREWS

BY

JOHN BASIL WILSON BSc.

Certificate.

I certify that John Basil Wilson, B.Sc., has spent nine terms on research work first under the direct: :ion of Professor R.C.Garry, M.B. D.Sc., and then under my direction and that he has fulfilled the conditions of Ordnance No. 16 (St.Andrews) so that he is qualified to submit the following Thesis in application for the degree of Ph.D.

Professor of Physiology,
University College,
Dundee.

Declaration.

I hereby declare that the following Thesis is a record of results of experiments carried out by me and that the Thesis is my own composition and it has not been previously presented for a higher degree.

The research work was carried out in the Physiological Laboratories of University College, Dundee, under the direction of Professor R.C.Garry, M.B. D.Sc., and of Professor C.H.Bell, B.Sc. M.D.

University and Research Training.

I entered University College, Dundee, in October, 1935 and graduated in June, 1940 with Second Class Honours in Chemistry.

In June, 1940 I was directed by the Ministry of Labour to Messrs. Imperial Chemical Industries Ltd., with whom I served until 1946.

In December, 1946 under the direction of Professor R.C. Garry I commenced the research work which forms the subject of this Thesis. Since October, 1947 Professor G.H. Bell has acted as my supervisor.

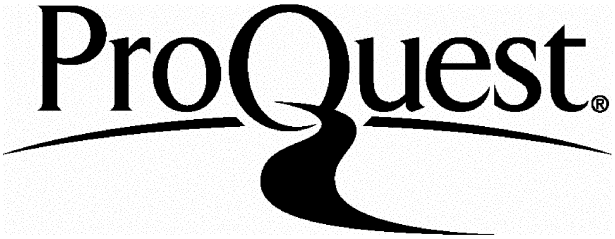
I was awarded a Carnegie Research Scholarship during the session 1947-48 and during the present session.

THE

ABSORPTION OF AMINO ACIDS

FROM THE

MAMMALIAN INTESTINE.



"The investigations of recent years dealing with the subject of intestinal absorption have, on the whole, tended to confirm the view that the membrane of the gut, provided that its normal condition is preserved, is not to be classed as a passive diaphragm between blood on the one side and solution in the gut on the other. Nevertheless, if the conclusion that the epithelium of the villi in some way acts as an active agent in absorption be admitted, we are still very ignorant regarding the extent to which its action is modified by changes in the physical and chemical environment of the cells within the limits of interference with bioplasmic processes, limits soon outstepped when concentrated solutions or solutions of deleterious substances are used by the investigator".

W. Waymouth Reid (1902),

J. Physiol. 28, 241.

CONTENTS.

Cats and of Cats under Urethane Anaesthesia.

GENERAL INTRODUCTION.

I. General nature of problem.

II. Structure and histology of the gut.

III. Historical review.

IV. Modern work.

V. Absorption of sugars.

VI. Absorption of fats.

VII. Absorption of amino acids.

VIII. Present position.

GENERAL INTRODUCTION.

a) General nature of problem:

Absorption is the term applied to the passage of substances into the blood stream, whether from the body surface, from the body cavities, the lymph spaces, or from any organs of the body. The absorption of foodstuffs takes place, in vertebrates, through the alimentary canal. Food is digested in order that it may be absorbed. The digested food thus diminishes in quantity as it passes through the alimentary canal, and the faeces contain the undigested or indigestible residue.

The alimentary canal shows considerable differences in structure along its length; these differences depend on the functions of the various parts. In the mouth and oesophagus absorption is at a minimum partly because of the thickness of the epithelium and partly because of the rapid passage of the food through these parts. Absorption takes place to a small extent only in the stomach. It is generally taught that water is not absorbed from the stomach (Hering, 1907). The most recent observations confirm the opinion that water is not absorbed in the stomach (indeed water provokes a secretion) and show that

alcohol is absorbed to some extent. Salts, also, do not seem to be absorbed unless present in much greater concentrations than occur in normal diets; sugar is absorbed with difficulty. Colloidal materials such as protein, fat, and starch, which are the main food substances apart from water and salts, are broken down in the intestinal canal by hydrolytic enzymes into water-soluble, diffusible substances (Starling, 1896). Thus proteins are broken down into amino acids or polypeptides, insoluble fats are broken down into fatty acids, which react with bile acids to form water-soluble, diffusible complexes, and starch is broken down into sugars.

This formation of water-soluble diffusible substances is not, however, the only significant result of the breaking down of foodstuffs. It is essential that the breakdown products should be non-specific substances. The importance of this can be seen with proteins. Proteins of one species of animal are toxic if injected into the circulation of another species, but the same protein taken into the intestine is split up into non-specific and non-toxic amino acids or polypeptides.

There are additional advantages in the breakdown of the foodstuffs. The body builds up in its cells substances which are specific to the animal,

and this is only possible if the food substances derived from plants or animals are first broken down to relatively simple molecules. Some substances such as water and soluble salts like sodium chloride are absorbed unchanged.

The small intestine, which possesses a very large internal surface on account of its folds and villi, is the main organ for absorption. Absorption begins in the duodenum, and the products of digestion have largely disappeared by the time the intestinal contents reach the ileocolic valve at the commencement of the large intestine; water is absorbed in the large intestine but its powers of absorption of other materials are very small. Absorption takes place chiefly in the upper part of the small intestine. After the food materials pass through the intestinal epithelium they reach either the blood vessels (portal tributaries) or the lymphatic vessels or lacteals. In general terms, the proteins and carbohydrates pass into the blood vessels, and the fats partly into the blood vessels and partly into the lacteals.

Thus, in the intestine, the foodstuffs are converted into forms which are not only diffusible, but also non-toxic, and capable of being used directly by

the organism or of reacting to form the characteristic substances of the body. The form in which the substance is present in the intestine is of decisive importance for the question of absorption. Diffusion is only possible in aqueous or lipoid solution. Certain of the digestive processes produce easily diffusible substances, e.g. amino acids in protein digestion.

It has been supposed that there must be processes which regulate the nature and amount of substances absorbed, in relation to the needs of the organism. But this has never been proved. Starling emphasised the fact that the normal intestine absorbs everything that is converted into a diffusible form in its lumen, independently of the needs of the body.

It is very difficult to find a truly physiological method for absorption experiments. The intestinal mucosa is extremely sensitive to alterations of temperature and blood flow. For example, the absorption of sugars from an isolated loop of the small intestine of the rabbit decreases if the temperature falls below 25°C (Auchinachie, Macleod and Magee, 1930). An abnormal permeability of the mucosa for raw protein has been seen when the pressure in the intestine is

increased through stasis, and a high intra-intestinal pressure due to injecting solutions into a ligatured loop of the intestine will cause abnormally rapid absorption (McDougall and Verzár, 1935). Movements of the villi cease if the blood flow becomes inadequate, and great structural changes, including bleeding or desquamation of the mucosa, may occur in response to disturbances in the blood flow.

The problem undertaken in the present work is the measurement of the rate of absorption of amino acids from the small intestine and the correlation of these results with the corresponding molecular weights and apparent molal volumes. For the study the two species, rat and cat, have been chosen.

b) Structure and histology of the gut:

The small intestine is especially adapted for absorption by its very large surface. Not only is its length very great but also its surface is increased many times by folds and even more by the intestinal villi. The small intestine is divisible into three portions called the duodenum, the jejunum, and the ileum. These three parts are not distinctly delimited, but gradually pass into one another. Their

structure, although showing some differences, is everywhere the same in principle, so that one description applies to all of them. The main functions of the small intestine are (a) the forwarding of the chyme along its course, (b) the continued digestion of the chyme by means of special juices secreted by its walls and by the accessory glands, and (c) absorption of the liquefied nutritive material into the blood and lymph vessels.

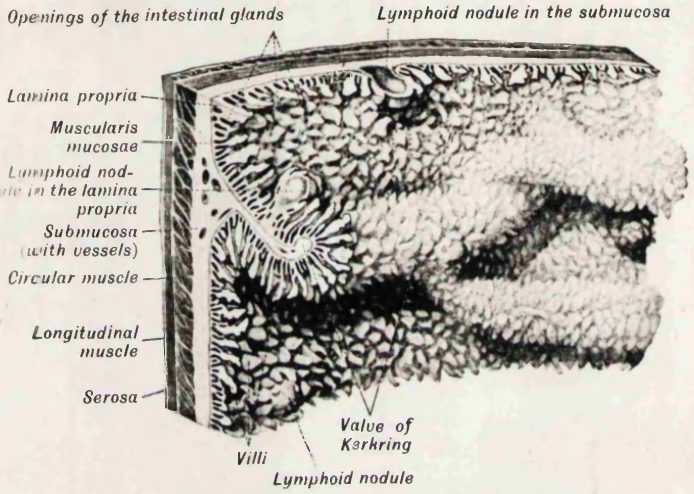
Surface of the mucous membrane:

The surface of the mucous membrane in the stomach, in which absorption is insignificant, does not show any outgrowths; in the small intestine, from which the organism receives almost all of its food material, the surface is enormously increased through the formation of circular folds, and the villi (Fig. 1). The folds are constant structures and do not disappear even when the intestinal wall is distended. They begin about 2 cm. from the pylorus and reach their maximal development in the distal half of the duodenum and the proximal part of the jejunum; in the ileum they become smaller and less numerous and disappear in its middle portion.

Figs. 1 and 2.

Fig. 1 is a stereomicroscopic view of the intestinal wall. Schematic. X 17.

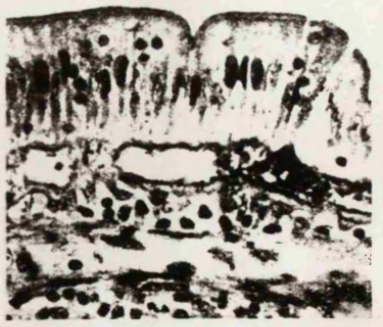
Fig. 2 is a section of a villus of the cat showing the close relationship of the blood capillaries to the epithelium. X 400.



Portion of wall of small intestine; drawn with binocular microscope and sections. 17 ×

after Braus, 1924.

Figure 1.



SECTION OF A VILLUS (CAT), SHOWING THE CLOSE RELATIONSHIP OF THE BLOOD-CAPILLARIES TO THE EPITHELIUM. × 400.

e, columnar epithelium-cells, with some leucocytes between them; *c*, blood-capillary cut lengthways; *l*, lacteal. Numerous leucocytes are also seen in the tissue of the villus and in the lacteal.

after Sharpey-Shafer, 1934.

Figure 2.

The villi are outgrowths of the mucous membrane covering the entire surface of the mucosa on the side and crests of the folds, as well as the spaces between them. They give the surface a typical velvety appearance. In the adult rat they are low, flat and leaf-shaped; in the cat they are more conical or finger-like (Figs. 3, 4, 5, 6 and 7). The innumerable openings of the intestinal glands or crypts of Lieberkühn may be seen between the bases of the villi (Fig. 7).

The epithelium:

The epithelium, which covers the free surface of the mucous membrane, the surface of the villi and small areas between their bases, is simple columnar. Three types of cells can be distinguished in it:- (a) simple columnar epithelial cells with a striated, cuticular border; (b) goblet cells; (c) chromaffine cells.

The common epithelial cells (Fig. 8) have a prismatic form; their outlines, however, change considerably with movement of the villi. The free surface is covered with a striated border and the lower part of the cell contains the oval nucleus. Under the striated border there is a thin layer of homogeneous protoplasm without any organoids or inclusions. The

Figs. 3 and 4.

Fig. 3 shows the histological appearance of the upper loop of the small intestine of the cat in transverse section after half isosmotic glycine had been left in the lumen of the gut for 40 minutes. The villi are long, finger-like processes. There is no apparent injury to the epithelial cells of the villi. X 6.

Fig. 4 shows the histological appearance of the upper loop of the small intestine of the cat after half isosmotic glycine had been left in the lumen of the gut for 40 minutes. The epithelium of the villi is absolutely intact. X 88.

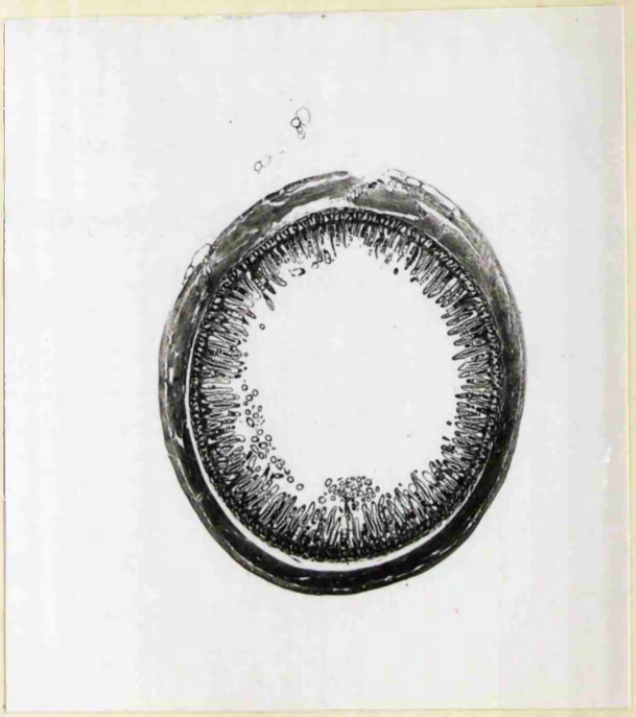


Figure 3.

Figure 4.

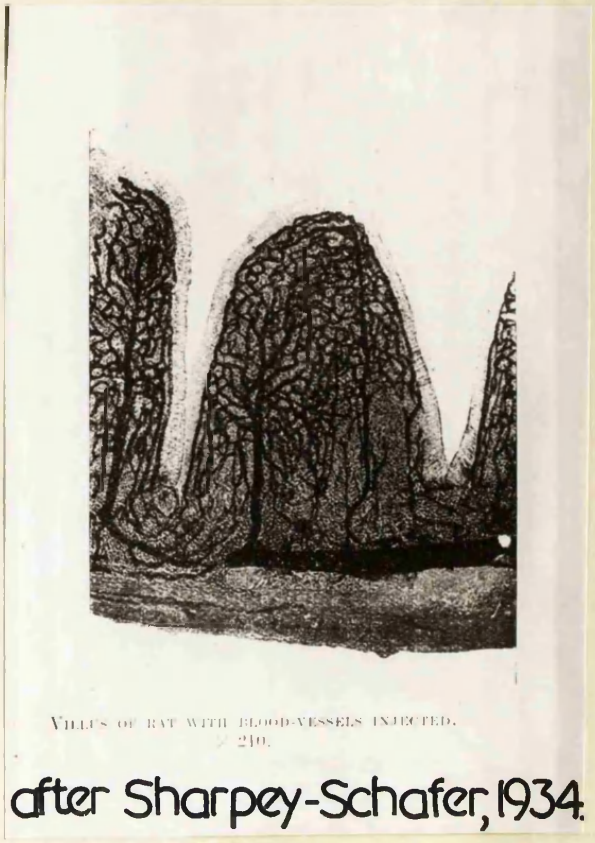
Figs. 5 and 6.

Fig. 5 shows the histolological appear:
:ance of a transverse section of a typical region of the upper loop of the small intestine of the rat after half isosmotic glycine had been left in the lumen of the gut for 1 hour. Since the villi of the rat are leaf shaped, in transverse sections they are rather like mounds than long processes.

X 14.

Fig. 6 shows the villus of the rat with blood vessels injected. X 210.

Figure 5.



VILLUS OF RAT WITH BLOOD-VESSELS INJECTED.
× 240.

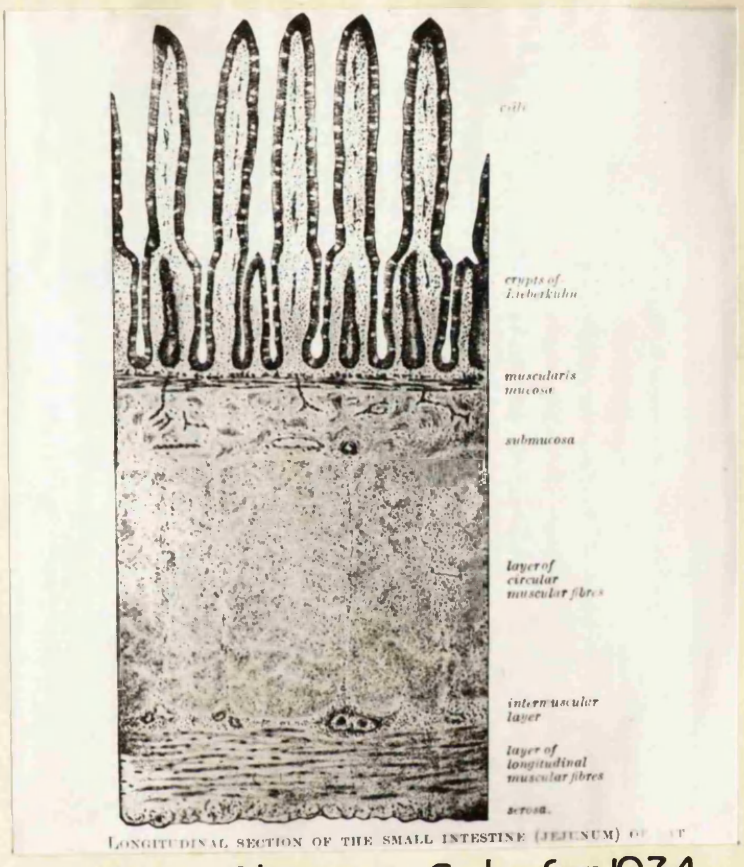
after Sharpey-Schafer, 1934.

Figure 6.

Figs. 7 and 8.

Fig. 7 shows a longitudinal section of the jejunum of the cat (semi diagrammatic) depicting the various coats of the small intestine. X 40.

Fig. 8 shows the columnar epithelial cells of the duodenal villi of the cat.



LONGITUDINAL SECTION OF THE SMALL INTESTINE (JEJUNUM)

after Sharpey-Schafer, 1934.

Figure 7.

after Mottram, 1922.

Figure 8.

close connection of the epithelium with the connective tissue, in the living condition, withstands successfully the strain arising from the movement of the villi and the mechanical action of the passing food material. In death, however, the connection is easily destroyed and it is extremely difficult to preserve the true intravital conditions in fixed material. After fixation the epithelium, as a rule, appears detached from the stroma on the summit of the villi in a continuous layer and a smaller or larger cavity is seen between the two tissues.

Goblet cells:

Goblet cells are scattered between the cylindrical epithelial cells. They occur on the free surface as well as in the crypts. They are single cells and their sides are convex by reason of the pressure of the mucigens and push away the neighbouring columnar cells.

Crypts of Lieberkühn:

The epithelium covering the villi continues into the glands of Lieberkühn. Above the bottom of the crypt their walls are lined with an undifferentiated, low columnar epithelium which contains numerous mitoses. Here regeneration takes place and the new cells moving upward gradually differentiate into goblet cells and into columnar epithelial cells with striated borders.

In the bottom of the glands of Lieberkühn in the small intestine, the peculiar, large cells of Paneth occur regularly. Their nuclei are large and rounded; their free edge cytoplasm is full of large spherical acidophilic granules, and their proximal cytoplasm contains basophilic iron - containing chromidial substance.

Chromaffine cells:

Chromaffine cells are the rarest. They are more abundant in the duodenum than either the jejunum or ileum and are found between the cells lining the glands of Lieberkühn.

In the middle of the villus is a lymphatic vessel, known as the central lacteal, which may be enlarged near its commencement; the enlargement is replaced in some animals by a network of small vessels. Surrounding the lacteal are fine bundles of plain muscular tissue prolonged from the muscularis mucosae. A network of blood capillaries (Figs. 2 and 6) lies quite near the surface under the basement membrane; it is supplied with blood by a small artery (in man and dog only one, in the rabbit, rat, cat, etc., two to three) which joins the capillary network at the base of the villus; the corresponding vein arises near the free

end of the villus.

The lamina propria of the mucous membrane fills all the spaces between the glands of Lieberkühn and forms the core of the villi.

Other coats of the wall:

The submucous layer consists of loose connective tissue. In the duodenum it is occupied by a thick layer of glands - the glands of Brunner which send their ducts to the inner surface of the mucous membrane between the crypts of Lieberkühn or into them.

The external longitudinal and the internal circular layers of the muscularis externa (Fig. 7) are well developed in the small intestine. The external coat consists of a layer of mesothelial cells resting on loose connective tissue, the serosa. At the attachment of the mesentery, the serous layer of the intestines continues on to the surface of the mesentery.

Absorptive activities:

The columnar cells bear the burden of the absorptive activities. The single layer of absorptive columnar cells is a border between tissue fluid within and the carefully selected, prepared, and regulated watery external environment supplied by the intestinal

contents. The epithelium of the small intestine admits water and mineral salts, the products of digestion of proteins, carbohydrates and fats.

The amino acids, which are split products of the proteins, are highly diffusible, enter quickly and cannot be observed in transit because our microchemical tests are not sufficiently delicate; alterations in cell structure have, however, been reported. Macklin and Macklin (1926) have shown vacuolation and swelling of the cells at the tips of the villi during protein absorption to be an agonal or early post mortem phenomenon.

After absorption the substances enter the tissue fluid on the other side and are taken up by the blood vessels and lymphatics. The capillaries form a very dense network in the lamina propria, or core, of the villus. The lymphatics here serve a double function. In addition to draining the tissue fluid, as they do elsewhere, they take up the absorbed fat; hence these structures are known as the lacteals. In the distended state these are the largest structures in the villi.

c) Historical review:

From the very beginning of physiological investigation the evolution of the conceptions of the mechanism of intestinal absorption has developed along

two main lines. It may be supposed either that absorption from the intestine is purely a physical or physico-mechanical process or that the process of intestinal absorption is, in part, at any rate, an active physiological function of the epithelial cells.

Longet (1868) quotes Hippocrates (B.C.460-377) and Galen (A.D. 130-200) as having propounded the earliest theories on the mechanism of absorption. The latter believed that absorption occurred through orifices in the blood vessels, particularly the venous blood supply, in the intestinal wall.

Rudolphi (1800) and Magendie (1825) were amongst the first workers to hold the view that physical "imbibition" was the cause of absorption. Also amongst the earliest recorded views on absorption was that of Tiedemann and Gmelin (1820) who compared the absorbing villi to inverted secreting glands. With the advance of knowledge concerning osmotic phenomena, Fischer (1822) and Dutrochet (1826) explained the process of intestinal absorption by the theory of endosmosis. Brücke (1851; 1852), however, still uphold the hypothesis formulated by Lieberkühn (1745) that the predominant factor in intestinal absorption was filtration. The theory demanded that intestinal peristalsis or contraction and expansion of the villi pressed fluid and solids into the

openings of the villi whence they were taken up by the blood vessels. The action was said to be one of filtration of the contents of the intestine by peristaltic pressure through the gut wall into the vessels of the villi. Both Lieberkühn and Brücke believed that they had observed openings into the villi. Obviously, such conditions would lead to obliteration of the capillaries and lacteals and, if filtration occurred at all, the fluids expressed would pass into the peritoneal cavity and only very slightly into the blood or lymph.

Voit and Bauer (1869) upheld this view following observations on the absorption of protein solutions, serum, and salts from the intestine. They concluded that filtration brought about by intra-intestinal pressure was responsible for intestinal absorption and not osmosis. These deductions were founded partly upon the erroneous idea that the passage of solutions through the intestinal wall should imitate the diffusion of the same solutions through artificial membranes. The fact that animal membranes may vary in their permeability was not known at the time.

It was not until 1881 that grave doubts were expressed by Hoppe-Seyler of the adequacy of a physico mechanical theory of filtration and osmosis to explain all the phenomena observed during intestinal absorption.

In fact, it seemed probable from the work of Ludwig (1851) and the investigations on the histology of the gut wall by Brücke (1854), Kolliker (1856) and Brettauer and Steinach (1887) that some special cell action was concerned in the process of absorption. Müller had, indeed, as early as 1841 declared that the cylinder cells discovered by Henle (1837) exerted in the process of absorption an "organishe Anziehung" (organic attraction). Hoppe-Seyler (1881) was the first to maintain definitely that intestinal absorption was a function of the living epithelial cells. The evidence was based upon the action of the gut in cholera and when poisoned by certain toxic substances. Since, in these cases, normal absorption did not occur Hoppe-Seyler considered that the "vital" activity of the intestinal cells had been impaired. In cholera, where the epithelium of the intestine is largely shed, the absorption is at a standstill although thinning of the membrane might favour osmotic transfer of the gut contents. Waymouth Reid (1900) pointed out that these experiments did not necessarily prove that the epithelium actively transferred the solutions into the blood in the normal state of affairs. He considered that the epithelium might act as "a barrier physically impermeable to certain substances in solution in the plasma and exerting osmotic pressure, and that with the removal of the barrier such substances can

diffuse over into the gut so that the value of their osmotic pressure as a factor in the absorption of water is annulled".

d) Modern Work:

Heidenhain (1894) and his pupils Leubuscher (1895), Cumilewski (1896) and Röhmann (1897) came to the same conclusion as that of Hoppe-Seyler, but with one modification. Heidenhain believed that a certain portion of the solvent and solute was absorbed by osmosis. Another portion was absorbed by the physiological activity of the living epithelial cells and did not obey the known physical laws. Heidenhain maintained that by the "activity of the living cell" the cell could exert an influence upon its physical or chemical processes.

Cohnheim (1898-1900) observed that the process of absorption was a one-way mechanism. His investigations led him to the decision that, under normal conditions, appreciable amounts of diffusible blood constituents did not pass into the intestine. Intestinal absorption, according to Cohnheim's theory involved two factors:-

(1) an impermeability to body fluids and dissolved substances due to the activity of the capillary endothelium which brought about the osmotic equilibrium between intestinal solutions and the blood without the passage of blood constituents into the gut solutions.

(2) an ability of the gut wall to take up the contents from its lumen through the intermediation of the vital activity of the epithelial cells, a function absolutely free of physical influences. This hypothesis, therefore, differed from that of Heidenhain in that it rejected the idea of cellular activity of the epithelium being affected by the osmotic pressure of the intestinal contents.

Waymouth Reid (1892-1902) on the same general grounds as Heidenhain and Cohnheim concluded that intestinal absorption was due to the physiological activity of the epithelial cells. He stated, however, that the forms of energy utilised in the cellular mechanism were not other than those known in the physical world. He assigned the power of absorption entirely to cellular activity and consequently his theory was in closer agreement with Cohnheim than with Heidenhain who postulated that osmosis played some part in the process of absorption. Waymouth Reid (1898 a,b.) based his conclusions on the evidence of a series of experiments where absorption of serum from the intestine under conditions in which the purely physical forces of filtration, osmosis, and adsorption were excluded. He showed that operations likely to favour the physical forces of filtration, osmosis, and adsorption such as injury to, or removal of, the

epithelium stopped this absorptive process. In fact, any condition depressing the activity of the cells tended to reduce the action of absorption. By means of a diffusion apparatus called an osmometer and an isolated surviving piece of intestine as a membrane Waymouth Reid demonstrated in 1892 that movement of substances took place across the membrane. The movement took place from the fluid in contact with the mucosa to that bathing the opposite serosal side of the intestine. Osmotic pressure influences be removed by using fluids of the same composition on either side of the membrane and in this manner, it was argued, the active force must be a vital function inherent in the epithelial cells. Waymouth Reid (1901 b) verified Cohnheim's finding that the process of absorption was a one-way mechanism. The reverse process of movement of substances into the gut lumen was said to result from cellular injury only, in which case the cells lost their "orienting action". In 1902 Waymouth Reid devised experiments to determine the effect of intravenous injections of sodium chloride on the absorption of glucose from the intestine. He showed that the degree of water passage from the intestinal solution was never increased under conditions of increased osmotic attraction of the blood, although in most cases there was a reduction in glucose absorption. Also, as the gut contents were found to contain no extra

chlorides above the normal amount he believed that the uptake of water from a solution in the intestine, where the epithelium was uninjured, was not a simple function of osmotic pressure of the sodium chloride of the blood. He concluded that as this was the chief blood salt it was not likely that any other blood constituent could influence the absorption of fluid from the intestine.

The work of these three authors provided the basis for the theory that the physiological activity of the intestinal cells was responsible for absorption from the intestine.

Hamburger (1896 - 1904) opposed the theory that the absorption process was due to "vital" cell activity. From a series of experiments intended to establish the effect of intra-intestinal pressure upon absorption he concluded that although physiological and pathological changes could affect the physical forces involved they did not cease to be purely mechanical. He found that increased intra-intestinal pressure facilitated absorption, a fact already established by Leubuscher (1885), and believed that the intra-intestinal pressure which would allow of absorption was lower than that of the intestinal capillaries, how much lower it might be depended on the force of the process of imbibition and the "sucking" action of the blood. In his mechanical theory Hamburger

also included the "suction" and pressure action of the villi as well as forces of osmosis and filtration. He claimed to have imitated all the phenomena exhibited in the intestinal absorption of sodium chloride and even of serum with artificially constructed membranes.

Höber (1898 - 1914), comparing the rates of diffusion of various solutions of salts with their speed of absorption in the intestine, concluded that although the physical factors play a part in the mechanism of absorption they did not fully explain the whole process. He was convinced that salts were absorbed into the intestinal cells only if they were lipoid soluble. This agreed with the work of Overton (1897; 1899), who postulated that lipoid solubility determined cell permeability. It was on this basis that Höber explained how the epithelial cells behaved towards dissolved substances like other cells. Dissolved substances having a parallel rate of absorption and speed of diffusion were lipoid insoluble and passed between the cells. Other materials having a greater rate of absorption than of diffusion were lipoid soluble and passed through the cells. Höber believed this theory to be not inconsistent or conflicting with any osmotic explanation of the process. This conception relegated a very insignificant role to the epithelial cells in the living animal and a predominant one to the intercellular spaces. Solubility

in lipoids unassisted by any other factor would, as Moore (1921) has stated, result in the accumulation to saturation point of the substances in question in the lipoids of the tissues and could not account for the steady stream of fluids through the epithelium that occurs during absorption.

Wallace and Cushny (1898; 1899) compared the rates of absorption of a large number of equimolecular salt solutions with a 1% (W/V) sodium chloride solution and found that the rates of absorption did not correspond with the diffusion rates. Salts which were easily dissociated into ions having great speed were not always absorbed faster than salts less easily dissociated and whose ions possessed lower speed. Wallace and Cushny declared that these facts did not necessarily indicate that the epithelial cells operated only by a "vital" activity and that it was more likely that the process of absorption was due to a chemical relation of colloid to salts of such a nature that the cell might take up one salt easily and reject another.

e) Absorption of sugars:

It was the careful study of the absorption of the monosaccharides which led eventually to the solution of the problem which has divided the mechanists and vitalists. Cori (1925) finally established the fact

that, although the known physical laws are factors of great importance in the process of absorption, they cannot explain all the phenomena concerned with absorption. Cori used unanaesthetised rats as his experimental animals. He determined the rates of absorption of monosaccharides from the small intestine by feeding a known amount of a given sugar in solution through a stomach tube, killing the animal, and estimating the amount of sugar remaining in the intestine. He was able to determine with great accuracy the amount of sugar absorbed in a given time by a rat of known weight. Within fairly wide limits, the amount of glucose absorbed did not vary with the concentration of the solute. In other words, the process of absorption did not conform to the laws of diffusion and osmosis. If absorption was merely a matter of diffusion the number of ions bombarding the absorbing surface in unit time would obviously be a factor of great importance.

Cori's method of determining the rate of absorption of monosaccharides suffered from one defect: namely the emptying time of the stomach was variable. To overcome this, Verzár (1935; 1936) injected the sugar solutions directly into loops of the small intestine of anaesthetised rats. The amount of sugar remaining in the loops, after a given period of time had elapsed was estimated. There is a remarkable agreement between

Verzár's and Cori's results. Both workers found that hexoses such as glucose and galactose were absorbed at a much faster rate from the small intestine of the rat than were pentoses such as xylose and arabinose. It was postulated by Verzár that the difference in the rate of absorption of hexoses and pentoses in the small intestine of the rat was due to a special activity of the intestinal mucosa, namely phosphorylation of the hexoses.

Evidence for this theory, however, is not conclusive and applies to the rat only. Verzár and Wirz (1937) postulated that the preferential absorption of glucose decreases as one progresses towards the caudal end of the small intestine of the rat and therefore the phosphorylating mechanism was more active in the cranial region of the gut. However, Davidson and Barry (1940) found that within the caudal half of the small intestine of the cat there was no evidence of decreasing absorptive power for glucose in the more distal region.

f) Absorption of fats:

The absorption of fat is slower than that for any other foodstuff. The main absorption of fat occurs inthe small intestine. The problem of fat

transport differs essentially from that of protein or carbohydrate transport in that the fatty acids, the elemental constituents of the fat, are insoluble in water as are also most of their compounds, while the corresponding elements of protein and carbohydrate are readily water-soluble.

There are three theories as to how fat, which is insoluble in water, can pass the epithelium of the mucosa, namely:(1) that fat might be dissolved in the lipoids of the cells after splitting into fatty acids, while the glycerol might be absorbed by its solubility in water (Heidenhain, 1888). However, fatty acids are practically not absorbed when introduced alone into an empty intestinal loop. Their lipoid solubility is far too slight to allow them to diffuse into the epithelial cells in any great quantity. (2) that fats are so finely emulsified by the bile that the fat droplets might be able to pass the epithelial wall and neutral fat appear again in the lymphatics (Munk, 1897). A mechanical force was once suggested to explain the entrance of these fine droplets into the epithelial cells but improved histological technique has shown this to be quite erroneous. (3) in 1901 Pflüger opposed this hypothesis, and put forward a new theory of fat absorption. He claimed that fat is saponified in the intestine, and is absorbed in the form of a water-soluble sodium soap.

The fats are split up by lipase in presence of bile into fatty acids and glycerol. Glycerol is water-soluble and diffuses readily, and the fatty acids are saponified by the sodium carbonate of the pancreatic and intestinal juices.

The main difficulty for the theory arose when the actual reaction of the intestine was tested. Pflüger speaks of the alkaline reaction of the intestine, but as a matter of fact, although the pancreatic juice has a pH of 8 or more, it is quickly changed by the intestinal contents. Kostyal (1926) studied the pH of the intestine of rats, dogs, guinea pigs and pigeons, especially for this purpose and found it to be seldom greater than pH7, and generally under this value, i.e. in the acid region. Robinson (1935) found a reaction of pH6.6 in the duodenum of rats, and pH7.5 - 8 at the ileocolic valve. This excluded the possibility that fats were absorbed in the form of alkali soaps.

According to Verzár (1936) the fatty acids combined with the paired bile acids to form compounds, which are not only water-soluble and diffusible, but also especially stable at the slightly acid reaction on the intestine. This bile-fat complex was broken down again in the mucosal cell. The bile acids were

adsorbed onto mucosal epithelium so that they were able to dissolve a much greater quantity of fatty acids than in vitro.

The fatty acid which was now in the cell combined with the glycerol, which was absorbed at the same time, to resynthesise neutral fat. This synthesis passed through an intermediate stage of phosphatide formation. The glycerol was phosphorylated and combined with the fatty acids. Thus the diffusion gradient into the cells for the fatty acids was increased. The synthesis of neutral fat, via the formation of phosphatide, was therefore an accelerating factor in the absorption of fat, just as the phosphorylation of glucose accelerated the absorption of this sugar.

In the mucosal epithelium the phosphatide was transformed again into neutral fat and, for the most part, appeared as such in the lymph. Significant but varying amounts escaped the transformation, and appeared in the lymph as phosphatide (lecithin), and so increased the lecithin content of the blood during fat absorption. There seemed to be no difficulty in explaining how the fat left the blood stream again, since the capillary walls have a permeability comparable with artificial membranes through which a great part of the serum-fat can diffuse out. (Verzár and Kuthy 1929 a,b,c; 1931).

It has been shown by Frazer and Stewart (1938) that a fat, such as triolein, was only partially digested in the intestine by the pancreatic juice. Hydrolysis determined the path taken by the absorbed fatty material, the fatty acid fraction passing by the portal vein to the liver, and the unhydrolysed portion by the lymphatic route into the systemic blood, and thence to the fat depots. This conception is contrary to the previously accepted hypothesis of Verzár. More recently, in a review of fat absorption, Frazer (1940) again mentioned that not all of the fat in the intestine was absorbed as fatty acids and glycerol, but that at least some of it was absorbed as emulsified fat, i.e. as tiny particles of fat or oil. He based his evidence in part on observations of the fat particles present in blood. These particles, called "chylomicrons" can be counted under darkfield illumination. An interesting argument in favour of the absorption of emulsified droplets of oil is the fact that paraffin oil is not ordinarily absorbed, but if it is emulsified by the addition of small amounts of oleicacid and cholesterol, then it is taken into the intestinal wall (Frazer, Stewart, and Schulman, 1942). Frazer and his co-workers were at a loss to understand how particles of fat or oil $\frac{1}{2}$ micron in diameter could find their way into the intestinal cells. However, Kopac (1937; 1940) and Kopac and Chambers (1937) have observed both for simple animal cells (e.g. sea urchin

eggs) and for plant cells that if an oily droplet is placed in contact with the cell membrane the oil will rapidly enter the cell interior. Emulsification of fats in the intestine is favoured by bile salts and these salts tend to make fatty acids more soluble. Thus the bile has an important influence on fat absorption.

(g) Absorption of amino acids:-

Present views on nature of amino acids when in solution: Since water undergoes ionisation with production of hydrogenion and hydroxylion, it will act both as an acid and as an alkali. This property is also met with in the case of many other compounds when dissolved in water, and which are therefore spoken of as amphoteric electrolytes or ampholytes. The amino acids are some of the most important of these.

Acetic acid, CH_3COOH, when dissolved in water undergoes ionisation to a small extend with production of hydrogen ions; it therefore acts as an acid. If the amino group ($-NH_2$) is introduced into the molecule, amino-acetic acid or glycine, $CH_2(NH_2)COOH$, is obtained; and this compound, in solution, cannot only yield hydrogen ion, but in virtue of the presence of the NH_2 group, can also, through reaction with water ($-NH_2 + H_2O = -NH_3OH$), give rise to hydroxyl ion. Glycine therefore behaves as an ampholyte and can act both as an acid (giving salts with

alkalis, e.g., $CH_2NH_2\ COONa$) and as a base (giving salts with acids, e.g., $CH_2\ NH_2\ COOH$, HCl). As in the case of all ampholytes, however, ionisation takes place only to a slight extent, so that glycine behaves as a very weak alkali and a very weak acid.

According to more modern views regarding acids and bases an ampholyte is a substance which can act both as a proton donor and as a proton acceptor. Thus in the case of aqueous solutions of glycine, $NH_2\ CH_2\ COOH$, one may have the reactions:

$$NH_2\ CH_2\ COOH \rightleftharpoons NH_2\ CH_2\ COO^- + H^+ \quad \text{or}$$

$$R \rightleftharpoons R^- + H^+$$

and $$HONH_3CH_2COOH \rightleftharpoons NH_3^+\ CH_2\ COOH + OH^- \quad \text{or}$$

$$R \rightleftharpoons R^+ + OH^-$$

Glycine may therefore yield both a cation and an anion.

Since an ampholyte can act both as an acid and a base it will have an acid ionisation constant K_a as well as a basic ionisation constant K_b (Walker, 1904 a,b.) The corresponding mass law equations are:-

$$K_a = \frac{[H^+]\ [R^-]}{[R]}$$ where R^- is the ion $NH_2CH_2COO^-$ or

$NH_3OH\ CH_2COO^-$ and R is NH_2CH_2COOH; and

$$K_b = \frac{[OH^-][R^+]}{[R]} \quad \text{or} \quad \frac{K_b}{K_w} = \frac{[R^+]}{[R][H^+]}$$ where R^+

is the cation $NH_3^+ CH_2COOH$. The constants K_a and K_b have been calculated from determinations of the degree of hydrolysis of the compounds of the ampholyte with strong acids and with strong bases, and from measurements of the conductivity of salts of the ampholyte. The values so obtained for several of the amino acids are very small, being about 10^{-9} - 10^{-10} for K_a and 10^{-11} - 10^{-12} for K_b. Since the amino acids may be regarded as substituted derivatives of acetic acid and ammonium hydroxide which have ionisation constants about equal to 1.8×10^{-5}, an acceptance of these low figures for the amino acids implies a tremendous influence of the substituent groups on electrolytic dissociation.

It was noticed by Bjerrum (1923) that by assuming the uncharged fraction of the amino acid to exist mostly in the form of doubly charged Zwitterionen or dipolar ions, (the word zwitter is the German term for a hermaphrodite), much more reasonable values for the constants of the amino acids could be obtained from the same experimental data. Therefore K_a refers to the dissociation of the NH_3 -group and the ionisation relations are described by the equilibria,

$$^+NH_3CH_2\,COOH \rightleftharpoons {}^+NH_3CH_2COO^- + H^+ \quad \text{or} \quad R^+ \rightleftharpoons R^{\pm} + H^+$$

and $H_2O + NH_2CH_2COO^- \rightleftharpoons {}^{+}NH_3CH_2COO^- + OH^-$ or $R^- \rightleftharpoons R^{\pm} + OH^-$

for which the mass law expressions are:-

$$K_A = \frac{[H^+]\ [R^{\pm}]}{[R^+]} \quad \text{and} \quad K_B = \frac{[OH^-]\ [R^{\pm}]}{[R^-]}$$

Evidently measurements which yield values for K_a of Walker's theory will give values for K_B of Bjerrum's theory and measurements, giving K_b will also give K_A. The two sets of constants may be related by identifying R and $R^{\pm}$, since each refers here to the total electrically neutral ampholyte. If the equations defining K_A and K_b are multiplied together, as are those defining K_B and K_a, it follows that $K_AK_b = K_BK_a$. These products are equal to the ion product of water since $K_w = [H^+][OH^-]\ Y_{H^+}\ Y_{OH^-}$ for any solution so dilute that the activity of unionised water may be taken as unity. In this and other mass law equations the brackets refer to the molal concentration, and Y to the activity coefficient of the ion or molecule. At very low concentrations all activity coefficients approach unity and activity tends to become identical with the molality.

The desired relations are, therefore, $K_A = \frac{Kw}{Kb}$ and $K_B = \frac{Kw}{Ka}$. The advantage of the Zwitterion system is that it gives more reasonable values to the constants, which are approximately $K_A = 10^{-2} - 10^{-3}$ and $K_B = 10^{-4} - 10^{-5}$.

In sufficiently acid solutions, the amino acid is positively charged and on electrolysis migrates to the cathode, whereas in sufficiently alkaline solutions it is negatively charged and migrates to the anode. Obviously there must be a definite hydrogen ion concentration at which it migrates neither to the anode nor to the cathode. This is called the isoelectric point. Each amino acid has a specific isoelectric point. So have all proteins. It was originally thought that at its isoelectric point, an amino acid was not dissociated either as an acid or as a base. According to the Zwitterion hypothesis, the amino acid molecule is electrically neutral at the isoelectric point, but this neutrality is due to the complete and simultaneous ionisation of acid and basic groups.

The fact that proteins consist of amphoteric amino acids is of great importance in interpreting many of their relations.

Höber and Höber (1936; 1937[a,b]) found amino acids to be absorbed at a higher rate than could be expected from their calculated molecular volume and even at a higher rate considering the volume of the zwitterions of the ampholytes being enlarged by a shell of water dipoles. On the other hand they discovered that by varying the concentration of

the amino acids introduced into loops of the small intestine of rats, the rate of absorption was not constant, but fell off with rising concentration. They concluded that amino acids resembled the "physiological" sugars (glucose, galactose) and were absorbed from the intestine by a selective process.

Evidence for physiological activity of the epithelial cells of the intestine during the absorption of amino acids is not so striking as in the case of the monosaccharides. Although his results were primarily the rates of absorption of sugars Cori (1926-1927) nevertheless found that the rate of absorption of amino acids also remained constant from hour to hour notwithstanding continual diminution of concentration. This suggested selective activity of the epithelial cells. These results were confirmed by Wilson and Lewis (1929) who based their work on that of Cori.

Nomenclature of the amino acids:

Until recently, the amino acids have been named with a prefix denoting the configurational family to which the α carbon atom belongs and the actual rotation indicated by a plus or a minus sign enclosed in parenthesis. Thus, we have $l(+)$ alanine and $l(-)$ leucine. This practice is often simplified and the amino acids referred to as l-amino acids and their enantiomers as d-amino acids, the above mentioned appearing as l-alanine and l-leucine. But this is very confusing with the early literature and

such reference works as Beilstein which names the amino acids with a prefix denoting the direction of rotation of an aqueous solution of the substance in the neutral range, e.g. d-alanine, l-leucine.

The new nomenclature agreed upon by the Biochemical Society and the Chemical Society in 1947 has been used throughout this thesis, namely, a prefixed small capital letter denotes the configurational family to which the α Carbon atom belongs; the optically inactive mixture or racemic compound is designated with the prefix DL; the actual direction of the rotation in a specified solvent, preferably of the free amino acid in water, is designated by a plus or a minus sign enclosed in parenthesis.

(h) Present position:

Even to-day, only the fringe of the problem of absorption of amino acids from the intestine has been tackled. Little is known about species difference in the rate of absorption of amino acids. Still less is known about the difference in the rates of absorption of the stereoisomers of a given amino acid in the same species. There is very little evidence to show if species difference exists between the rates of absorption of the stereoisomers of an amino acid. This thesis is an attempt to investigate one or two of the outstanding problems in the field of absorption of

amino acids from the small intestine.

---oOo---

SECTION "A".

RATES OF ABSORPTION OF AMINO ACIDS FROM UPPER AND LOWER LOOPS OF THE SMALL INTESTINE OF RATS.

I. Introduction.

II. Past work.

III. Present work.

a) Method.

b) Results.

c) Discussion.

IV. Summary.

---oOo---

Rates of Absorption of Amino acids from upper and lower loops of the small intestine of Rats.

I. Introduction:

Within recent times there has been a renewal of interest in the problem of absorption of amino acids from the intestinal tract. The straightforward rate of absorption of amino acids has a bearing on the present use of amino acids mixtures in therapeutics; in certain conditions, these have been given in preference to the more usual forms of protein food. It is obviously of moment to know the relative rates of absorption of the different amino acids.

A survey of the literature showed that some data existed for the absorption rates of glycine and alanine from the small intestine of the rat. Much of this work, however, is in need of confirmation. No data could be found for the absorption rates of phenylalanine and isoleucine from the small intestine of the anaesthetised or decerebrate rat. It seemed worth while, therefore, to try to obtain figures for the absorption rates of these substances from loops of the small intestine of the anaesthetised and of the decerebrate rat. No data is available in the literature for absorption rates of amino acids from the small intestine of the decerebrate rat. It was also considered profitable to compare the relative rates of absorption of each amino acid in the upper and lower regions of the small intestine.

II. Past Work:

Breakdown of protein:

All research on the absorption of proteins before 1900 was done in ignorance of the fact that protein is broken down to amino acids in the intestine. The important workers in this field were Brücke (1851), Voit and Bauer (1869), Friedländer (1896), Neumeister (1897), Czerny and Latschenberger (1874), and Heidenhain (1888).

The first clearly defined theory of protein absorption was that enunciated by Liebig (1856) who assumed that protein material undergoes little or no chemical change previous to its introduction into the blood stream and its assimilation by the tissues. He believed that the only necessary preparation was that of putting the proteins into solution in order that they might be absorbed, and this was the sole object of digestion. In support of this view the fact was demonstrated that unchanged proteins, e.g., serum and uncoagulated egg albumin (Voit and Bauer (1869), Heidenhain (1894), Friedländer (1896), Waymouth Reid (1900) and others, pass directly from the alimentary canal into the blood.

More thorough investigation has shown decisively, however, that the absorption of unchanged proteins is an ab: :normal process. A knowledge of the proteolytic powers

of the digestive tract was first attained by the famous experiments of Spallanzani (1780) and Beaumont (1833) with the gastric juice. The identification of trypsin by Kühne (1867), and the discovery by Cohnheim (1901) of the intestinal enzyme erepsin which splits up peptones and proteoses into amino acids initiated a new period of research.

It was not until the experiments of Salvioli, Hofmeister, and others were carried out that the old theory, advanced by Leibig, was finally abandoned and a new explanation of the mode by which the proteins were absorbed from the small intestine sought. Salvioli (1880) and Hofmeister (1882) found that the stomach wall of the digesting dog contained peptone which disappeared rapidly or at any rate was so altered that it no longer gave the reactions by which it was characterised. Hofmeister pointed out that the disappearance could be due to either re-synthesis into protein or further digestion of the peptone. Heidenhain (1888) repudiated the theory of resynthesis but afterwards, like Shore in 1890, he inclined to the view that the peptone was converted into protein, and that the epithelial cells of the small intestine played an important part in this change.

In 1897 Neumeister found in the intestinal mucosa two decomposition products of protein, leucine and tyrosine,

which suggested that further breakdown had taken place. This observation had already been made some forty years previously by Kölliker (1856) and Müller (1841) who concluded that the leucine and the tyrosine were either absorbed or broken down further as they were unable to find them in the faeces. The presence of these amino acids, however, did not necessarily exclude the possibility of a subsequent synthesis. In 1867 Kühne, who gave us the terms "trypsin" and "enzyme" carried out his classical experiments on pancreatic digestion. He found that the residue of protein, which is unaffected by pepsin, is attacked by trypsin and gives rise to "tyrosine-leucine", a mixture of amino acids, and complex substances over which trypsin has no digestive power. Kühne rightly described the tyrosine and leucine as being products arising from the breakdown of protein, but thought them normal digestion products on the way to absorption. Schmidt-Mulheim (1879) repeated the work of Kühne and concluded that the breakdown which occurred was unimportant. Sheridan Lea (1880) agreed with Kölliker and Müller.

Salkowski and Leube (1880) suggested that the leucine should be considered as a product which after absorption was used for rebuilding purposes, and which therefore could be regarded as a stage towards protein regeneration, analogous to the conditions ruling in plant physiology where it had been demonstrated that the decom-

:position products of protein, asparagine, leucine and tyrosine, could be regenerated into protein when carbohydrate was also present. Kutscher and Seemann (1901), as a result of their investigations on the fate of protein in the small intestine of dogs considered the above hypothesis feasible.

Absorption of proteins as amino acids:

In 1901, however, new light was thrown upon this problem by the important work of Cohnheim. He fed meat to a dog with a duodenal fistula which permitted isolation of the products of digestion after they had passed the stomach and part of the intestine. The partially digested products obtained from the fistula were treated with erepsin for 24 hours, the action of this enzyme thus following, as in normal digestion, that of the gastric and pancreatic juices. The meat proteins were hydrolysed completely. The presence of peptides or intermediate products could not be detected. Thus an increase of protein, i.e., a regeneration, did not take place. Cohnheim postulated that normal digestion proceeds in the intestinal lumen and wall until most, if not all, of the proteolytic products are reduced to the stage of free amino acids. These are absorbed into the blood stream, from which they rapidly disappear as the blood circulates through the tissues.

Abderhalden (1912a) showed that all the known amino acids were present in the mammalian intestine during

the absorption of protein; this work was subsequently confirmed by Cohnheim (1912; 1913). Abderhalden (1912b), in his book "Synthese der Zellbausteine" supposed that the intestinal mucosa synthesises proteins again from the amino acids. Zunz (1908) and Körösy (1911) were of the same opinion. They had no efficient method to show the increase of amino acids in the blood during absorption and therefore postulated a synthesis of protein from the amino acids which disappeared from the intestine. Later, Abderhalden found positive evidence that intact protein was not normally absorbed into the circulation. Gayda (1915) working with artificially perfused intestines suggested that some complexes of amino acids were formed or some amino acids were selectively absorbed. London and Kotschneff (1934) also stated that there was no proof of protein building up in the intestinal mucosa.

From the work of Furth and Friedmann (1908) and more especially of Abderhalden, with Körösy and others (1907) it was postulated that the amount of amino acids decreases towards the ileal end of the small intestine. Abderhalden and London (1910) introduced a mixture of amino acids from digested protein into the intestine, and found that about 80% was absorbed after 3 hours. All the evidence seems to show, firstly, that protein is broken down to amino acids, and secondly, that amino acids are quickly absorbed from the intestine.

Concentration of amino acids in the blood during absorption:

Another method used to prove that proteins are absorbed as amino acids was to try to show that the concentration of amino acids increased during protein absorption (Cathcart, 1912). However, as late as 1905, Kutscher and Seemann were unable to identify the presence of any amino acids in the blood of dogs during protein absorption, even when they excluded the liver and kidneys from the circulation to increase the concentration. But in the next year Cathcart and Leathes after injecting proteins into the intestine found in the blood and in the liver an increase of nitrogen products which we now know must have been amino acids. In 1910 Delaunay found the amino nitrogen content of both arterial and the portal venous blood to be 9.9 and 21 mg.% respectively. Following either a meat meal or the injection of a solution of amino acids into the intestine he found (1913a,b,c) there was a definite rise in the blood content of amino acids most marked in the case of the portal blood.

Van Slyke and Meyer (1912) and Folin and Denis (1912: a,b,c,d,e) found during the absorption of meat and other proteins that amino acids increase in concentration in the blood. Injecting amino acids into the intestine they found gave an increase in the blood amino nitrogen content.

The great difference between the amino acid content of arterial and portal blood proved that it was the liver which retained the amino acids for further synthesis and breakdown. Even in the fasting state the arterial blood contains a small amount of amino acids but this is due to amino acids always being transported (from the liver, for example) to different organs. Muscle tissue takes up amino acids from the blood.

The liver has great capacity for retaining amino acids. During absorption the quantity of amino acids therein may increase 150%. This great accumulation of amino acids in the liver explains why the concentration of amino acids in the blood increases so very slightly during intensive absorption of protein from the intestine (Van Slyke, 1917).

Van Slyke (1912) demonstrated the rapidity with which amino acids leave the blood. When he injected alanine intravenously into a dog, only 12.5% was found in the blood after 5 minutes, and after 35 minutes only 3.5%. In the same period only 3.5% was excreted by the kidneys, so that the cause of the decrease must be sought elsewhere. One volume of blood flowing through the bowel is so large that an increase of only 3 mg.% is enough to account for the total quantity of protein absorbed. The rapid disappearance of amino acids from the blood explains why the increase of

amino acids in the blood is so slight that past workers were unable to show it. It was not until the more sensitive methods of Van Slyke (1911; 1912; 1913-1914 a,b) (1915; 1929) for the estimation of small amounts of amino acids in the blood were developed, that these problems were solved.

Abel (1913) and Abel et al (1913-1914) by his method of vivi diffusion in which a large fraction of the animal's blood could be passed through a dialysing apparatus and then returned to the body after having lost its diffusible contents, showed that amino acids could be isolated in considerable quantity from the dialysate. The portal venous blood contained considerably more amino acids than that of the carotid. That amino acids existed in the blood stream has received repeated confirmation. Thus Abderhalden (1913) obtained a great variety of amino acids from a large quantity of blood by precipitation methods.

It is significant that the increase of amino acid concentration in the blood goes parallel with the rate of absorption of proteins. Kuthy (1930) measured the rate of absorption of 7g. raw meat from rats and showed that at the end of 3 hours it reaches its highest value when 50% of the meat has been absorbed. From the third to the sixth hour absorption decreases to 30% and the amino acid content also slowly decreases. When in the sixth to ninth hour absorption

Fig. 9.

Fig. 9 gives a graphical picture of the conditions of protein absorption. It shows that the proteins are absorbed more slowly the longer absorption has proceeded; that the increase of amino nitrogen in the blood runs parallel with the absorption; and that an increase in oxygen consumption also goes parallel with the absorption of protein.

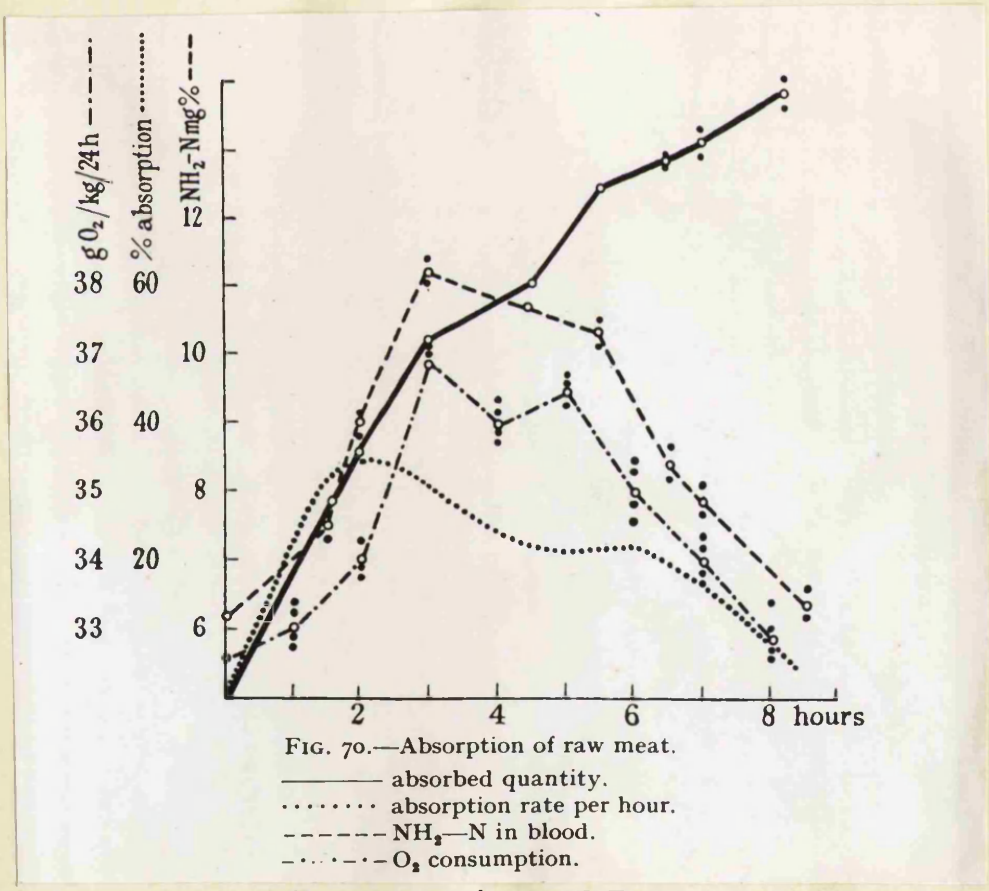


FIG. 70.—Absorption of raw meat.
——— absorbed quantity.
····· absorption rate per hour.
– – – – – – NH_2—N in blood.
– · · · – · · · – O_2 consumption.

after Kuthy, 1930.

Figure 9.

decreases to 10% and less (Fig. 9), the amino acid value returns to normal. After 9 hours 90% of the protein had been absorbed. The amino acid content of the blood gives a true picture of the rate of protein absorption. At the same time the oxygen consumption (amino acids stimulate oxygen consumption) is exactly parallel, reaching highest values at the same time as the amino acid concentration of the blood. The absorption rate, calculated from the disappearance of nitrogen from the intestine, reaches its maximum just before the blood amino nitrogen.

Verzár and McDougall (1936) described the conditions of protein absorption. These were, firstly, proteins are absorbed more slowly the longer the absorption has proceeded. This may be explained by the constant decrease in concentration in the intestine. Secondly, the increase of amino nitrogen in the blood runs parallel with the absorption. Thirdly, the increase of oxygen consumption goes parallel with absorption of protein.

The discovery that protein is hydrolysed to amino acids in the small intestine, and is absorbed and transported in this form must inevitably be followed by an intensive study of the behaviour of the individual amino acids, if a true picture of protein metabolism is to be obtained. This shift in viewpoint has tended to simplify the study of protein metabolism since the various proteins present,

when hydrolysed, essentially the same qualitative picture. The problem thus is altered from a consideration of many compounds (the individual proteins) to a consideration of relatively few (the amino acids).

Rate of absorption of individual amino acids:

One of the earliest suggestions that the rate of absorption from the intestine might not be the same for all amino acids came from indirect evidence. Seth and Luck (1925) stated that the amino acid nitrogen of the blood of dogs and rabbits showed a more rapid increase after oral administration of glycine and alanine than after similar administration of glutamic and aspartic acids. They suggested that the di-carboxylic amino acids might be less rapidly absorbed. In 1928 Johnston and Lewis, who fed amino acids to rabbits and studied the changes of the non protein nitrogen of the blood, confirmed the work of Seth and Luck. They believed the rate of absorption of amino acids from the intestine to be an important factor in the rate of metabolism of the amino acids. Wilson and Lewis (1929) extended the preliminary experiments of Cori (1925; 1926-1927) who studied the absorption rates of glycine and alanine in rats, to include glycine, alanine, glutamic acid and leucine. The absorption of most of the other amino acids of the protein molecule has now been investigated by the Cori technique or a modification of it.

Wilson and Lewis (1929) fed rats with different amino acids and confirmed Cori's findings that the rate of absorption was not influenced by the absolute quantity of amino acids in the intestine. They stated that the rate was correlated to the amino nitrogen content of the blood and claimed there were no significant differences between the rates of absorption of naturally occurring optically active and synthetic racemic forms of the amino acids. Wilson and Lewis arranged the amino acids they investigated in the following descending order of rate of absorption:-
L alanine, DL alanine, glycine (Na), L glutamic acid (Na), glycine, DL alanine (Na), L leucine (Na).

Cystine (Sodium salt):

Stearns and Lewis (1930) studied the absorption of cystine (in the form of the sodium salt) from the small intestine of rabbits, using the method of Cori. They concluded that the compound was not absorbed rapidly. Wilson (1930) measured the rate of absorption of cystine (in the form of the sodium salt) from the small intestine of the rat. When fed as the hydrochloride the cystine was absorbed still more slowly. Sullivan and Hess (1931) repeated Wilson's work on cystine to compare the Sullivan (1929 a,b) method with the Folin Marenzi (1929) colorimetric method of determining cystine which had been employed by Wilson. They checked their results by the Okuda iodometric

(1925) method and verified Wilson's findings.

Andrews and Johnston (1933 a,b) studied the absorption rates of L and DL cystine, cysteic acid, and sodium sulphate from Thiery loops in dogs. In 1935 and 1936 Andrews, Johnston and Andrews reported similar but more detailed studies applied to the absorption of cystine, methionine and cysteic acid. Cysteic acid was absorbed most rapidly, while L cystine and sodium sulphate were absorbed most slowly.

Tryptophan:

Berg and Baughers (1932) undertook similar studies of the rates of absorption on tryptophan and tryptophan derivatives, and confirmed the results of Cori (1926-27) and of Wilson and Lewis (1929) for glycine. They observed the following descending order of rates of absorption: acetyl DL tryptophan, acetyl L tryptophan, L tryptophan, DL tryptophan, DL tryptophan ethyl ester and L tryptophan ethyl ester.

Leucine and Valine:

Chase (1933) working with Lewis (1933; 1934) determined the rates of absorption of several amino acids, in the form of sodium salts, from the small intestine of the rat. Chase found no difference between the rates of absorption of isoleucine and norleucine, but isovaline was

absorbed much more slowly (as its sodium or potassium salt) than valine, although at the same rate if given in the free form.

Höber (1936a) observed that the rate of amino acid absorption from the small intestine of rats was too fast to be explained by diffusion. Höber and Höber (1936; a,b; 1937) suggested that a cellular mechanism was involved in the preferential absorption of amino acids. Amino acids exhibited irregular behaviour and there seemed to be no relation between size of molecule and the rate of absorption. However, this work with rat intestinal loops did not compare amino acids among themselves.

Lysine, Histidine and Arginine:

Working with rats, Doty and Eaton (1937 a,b; 1938) reported individual variations in the absorption rates of L lysine, L histidine and L arginine (but not of glycine) which appeared to be correlated with the amount of amino acid fed. They concluded that in the case of these three amino acids the absorption rate was a direct function of the amount fed, and that the rate increment per unit increase in dosage was greatest with histidine and least with lysine. Arginine occupied an intermediate position. Their experiments corroborated those of Wilson (1930) and Wilson and Lewis (1929) in demonstrating that the quantity of glycine fed had no appreciable effect upon

the rate at which it was absorbed from the small intestine of the rat.

Remmert and Butts (1942) were unable to agree with the rate of absorption of L histidine reported by Doty and Eaton, and claimed that the absorption rate was very much slower.

Glycine, Alanine and Serine:

Sarzana (1933) measured the rates of absorption of different amino acids from Vella-fistulae of dogs. He found that while serum protein was practically unabsorbed in 1 hour, amino acids were absorbed at rates varying from 16% to 72% of the original amount introduced in half an hour. Alanine was absorbed most quickly, then glycine, then glutamic acid, and finally, aspartic acid.

Gallo and Alieri (1938) claimed that mixtures of peptone and amino acids were each absorbed in proportion to their own concentration. Althausen (1939) reported that the rate of absorption of alanine from the small intestine of rats was greater from a 10% than from a 5% solution.

In 1938 Laszt described how the rate of absorption of glycine, but not of certain other amino acids, from the small intestine of rats was reduced to about half by extirpation of the adrenals. He observed the following descending order of rates of absorption in the normal rat:

glycine, DL serine, DL alanine, L iso leucine and L valine.

Lathe (1943) concluded from his work with intestinal loops in dogs that the rate of intestinal absorption of amino acids decreases with an increase in their molecular size. Mehl and Schmidt (1937) found that the diffusion coefficient of amino acids in aqueous solution was related to the size and shape of their molecules, rather than to their molecular weights. Kratzer (1944) reported that the rates of absorption of various amino acids from the small intestine of the chick varied inversely with the apparent molal volume of the amino acid (Fig.10).

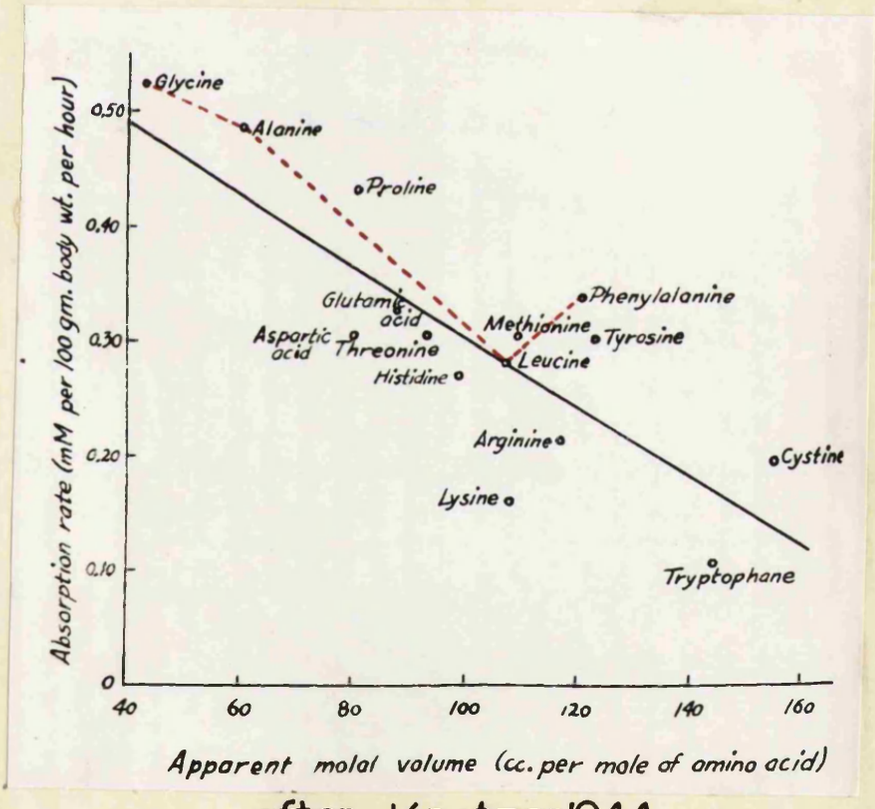
Amino acid derivatives of propionic acid:

The rates of absorption of certain amino derivatives of propionic acid from the small intestine of rats were studied by Schofield and Lewis in 1947. They arranged the acids studied in the following descending order of absorption coefficients: L-and DL-alanine (essentially the same), DL-serine, D-alanine, β-alanine and DL-isoserine.

Table I summarises the data on the rates of absorption of amino acids from the small intestine.

Fig. 10.

Fig. 10 shows the relationship between the millimolar rate of absorption of an amino acid from the gastro intestinal tract of the chick and the apparent molal volume of the amino acid.
The red dotted line joins points plotted by Kratzer for glycine, alanine, leucine and phenylalanine. The amino acids employed in this thesis are glycine, alanine, isoleucine and phenylalanine. The red dotted line corresponds exactly in shape to the graphs in Fig. 17 drawn for absorption values measured in the rat.



after Kratzer, 1944.

Figure 10.

-TABLE I.

Relative Rates of Absorption of amino acids from the small intestine.

Author.	Animals.	Relative Rates of Absorption.
Seth and Lack (1925).	Dogs.	Alanine and glycine > glutamic acid and aspartic acid.
Cori (1926-27).	Rats.	DL-Alanine > glycine.
Johnston and Lewis (1928).	Rabbits.	Glycine= DL-alanine > L-glutamic acid.
Wilson and Lewis (1929).	Rats.	L-Alanine > DL-alanine > glycine(Na) > L-glutamic acid (Na) > glycine > DL-alanine (Na) > L-leucine (Na).
Wilson (1930).	Rats.	L-Cystine (Na) > L-cystine hydro: :chloride.
Berg and Banghera (1932).	Rats.	Acetyl DL-tryptophan (Na) > acetyl L-tryptophan (Na) > L-tryptophan (Na) > DL-tryptophan (Na) > glycine > ethyl ester DL-tryptophan hydro: :chloride > ethyl ester L-trypto: :phan hydrochloride.
Wilson(1932).	Rats.	L-Alanine > glycine.

TABLE I. (Contd).

Relative Rates of absorption of amino acids from the small intestine.

Author.	Animals.	Relative Rates of Absorption.
Lawrie (1932).	Rats.	DL·Cystine > L· cystine.
Chase (1933).	Rats.	DL·Isovaline > DL·isovaline(Na) L·Valine(Na) = DL·valine(Na) > L·Leucine(Na) > D·leucine(Na)
Sarzana (1933)	Dogs.	L·Alanine >glycine >L·glutamic acid. > aspartic acid.
Andrews and Johnston. (1933 a,b).	Dogs.	Cysteic acid > DL·cystine > L·cystine.
Chase and Lewis. (1933; 1934).	Rats.	L·Valine(Na) = DL·Valine(Na) >L·leucine(Na) = DL· leucine(Na). >L·isoleucine(Na) = DL· isoleucine (Na) = DL·norleucine (Na) > DL·isovaline (Na or K).
Andrews, Johnston and Andrews (1935;1936).	Dogs.	Cysteic acid > cysteic acid hydantoin. > cystine phenylhydantoin > dibenzyl cystine > L·cystine. > cystine hydantoin.

TABLE I. (Contd).

Relative Rates of Absorption of amino acids from the small intestine.

Author.	Animals.	Relative Rates of Absorption.
Höber and Höber.	Rats.	Glycine > L-alanine > L-valine > L asparagine.
Doty and Eaton. (1937a, b; 1938).	Rats.	L-Histidine hydrochloride > Arginine > L-lysine hydrochloride.
Laszt (1938).	Rats.	Glycine > DL-serine > DL-alanine > L-isoleucine > L-valine.
Althausen (1939).	Rats.	L-Alanine (varies directly with the concentration).
Lethe (1943).	Dogs.	Glycine > DL-alanine > L-norleucine.
Kratzer (1944).	Chicks.	DL-Phenylalanine (Na) > L-tyrosine (Na) > L-proline (½Na) > L-cystine (Na) > DL-methionine (Na) = L-glutamic acid (½Na) > DL-alanine (½Na) > L-histidine hydrochloride > L-aspartic acid (½Na) > glycine (½Na) > L-arginine hydrochloride = L leucine (Na) > DL-threonine (½Na)

TABLE 1. (Contd).

Relative Rates of absorption of amino acids from the small intestine.

Author.	Animals.	Relative Rates of Absorption.
		DL lysine (HCl) DL tryptophan (Na).
		Glycine gives the same absorption rate at all concentrations.
		Tyrosine and arginine (vary directly with the concen: :tration).
		Glutamic acid (varies inversely as the concentration).
Schofield and Lewis. (1947).	Rats.	L Alanine - DL alanine
		DL serine.
		Dalanine alanine.
		DL isoserine.

----oOo----

III. PRESENT WORK.

a). METHOD:

Operative procedure:

The method was basically that of Cori (1925). A known quantity of a solution of amino acid of given strength was placed in the gut and the residual quantity determined after a time interval. Since there is little doubt that the previous history of the animals may effect the absorbing power of the gut (Westenbrink, 1934) all the rats were kept on a complete stock diet of rat cubes (Thomson, 1936) milk and greens. For the 24 hours previous to the experiment the rats received only water.

They were then given a subcutaneous injection of a freshly made 25% w/v urethane (ethyl carbamate) solution. The dosage was 1.6 mg. urethane per gramme rat. In some cases it was necessary to increase the dose slightly to obtain the required depth of anaesthesia. Sufficient depth of anaesthesia was usually reached an hour after the injection of urethane. During this period the rat was kept in a cage over a warm radiator in order to ensure that its temperature remained constant at 38°C; otherwise the animal died. Rats under urethane anaesthesia seem to be very susceptible to changes in

environmental temperature. It was found at this stage that if their temperature rose above 38° - 39°C, the animals suffered from hyperthermia and showed signs of cyanosis and respiratory distress. Death very often ensued. Urethane in smaller dose was the anaesthetic used by Verzár and McDougall (1936) whose absorption values of sugars from the small intestine in normal rats agree well with those of Cori (1925) on unanaesthetised rats. The dose of urethane employed by Verzár, 1.2 mg. urethane per gramme rat was found to be in-sufficient to induce full anaesthesia. It has been shown that urethane has a comparatively slight inhibiting influence on the absorption of water from the gut (Heller and Smirk, 1932).

The abdomen was opened approximately one hour after the injection of urethane and the small intestine exposed.

A ligature A (see figure 11) was tied round the extreme upper end of the small intestine. A second ligature B was placed at a point 3 cm. caudal to A, and the ends left untied. A snip, sufficient to accommodate a cannula, was made between A and B. To ensure approximately equal lengths of the upper and lower loops of the small gut, a piece of wet thread 30 cms. long was used to measure off a length of gut from B. A ligature

G, similar to B, was placed round this point which was approximately 30 cms. caudal to B. Similarly, beginning at the caecum, ligatures E and F were placed round the ileum 3 cms. apart E being tied but F left loose. A ligature D, correspon: :ding to A, was tied at a point approximately 3 cms. caudal to G. A snip through the gut wall was made between G and D. 30 cms. of gut were measured with the thread from F to G. Ligatures G and H were placed 3 cms. apart H being tied but G left loose, and a snip through the gut wall made between them. A snip big enough to admit a cannula was made through the gut wall between E and F. A cannula was then placed in the openings between A and B and E and F. All faecal matter was washed out with warm saline (1% w/v) at 37 C. Excess saline was removed by puffing air very gently through the lumen of the gut.

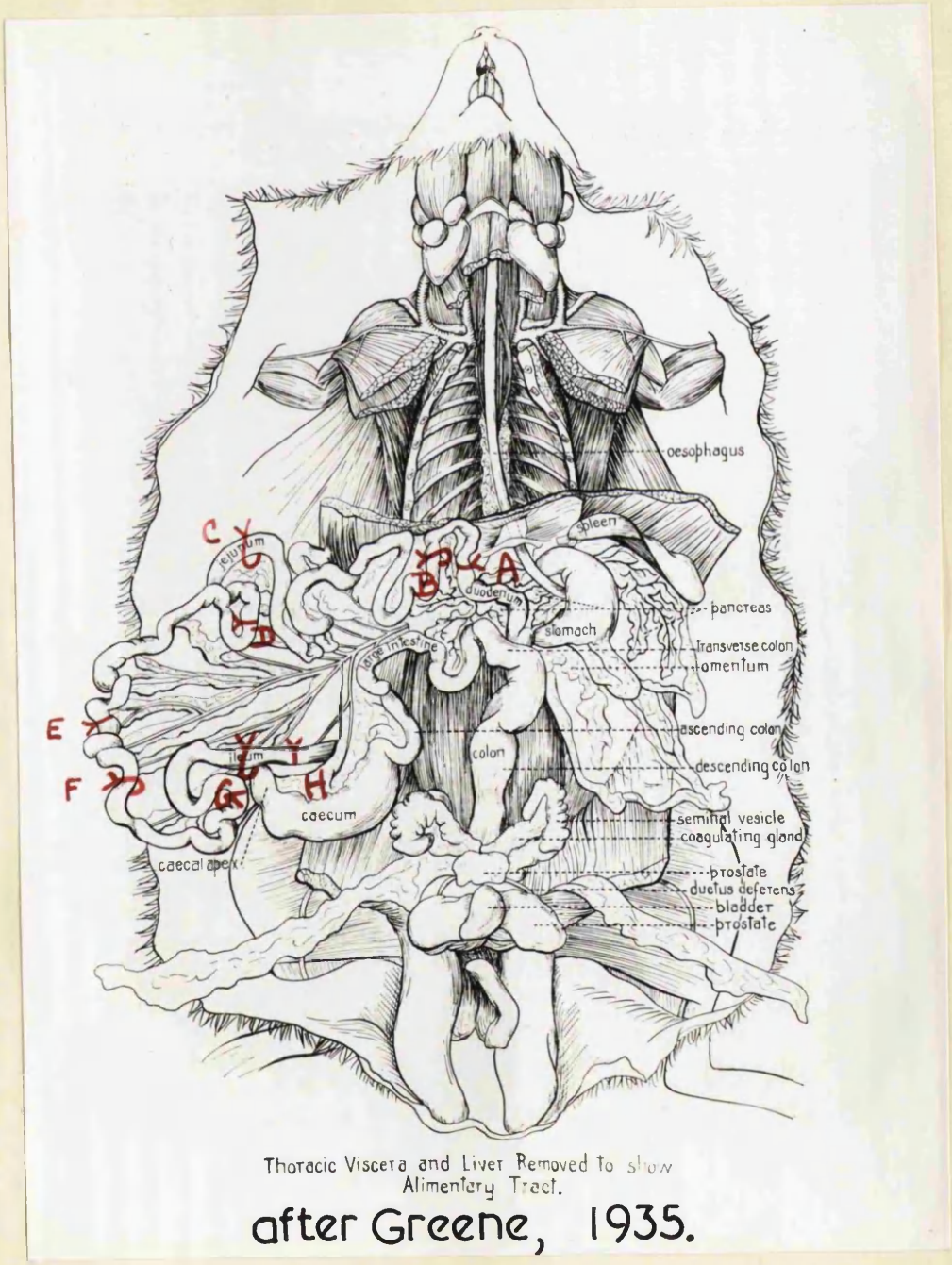
The loose ligatures G and G were then tied off. 1.5 - 2.0 c.c. of an isosmotic or half isosmotic amino acid solution at 37 C were run into each loop by means of a cannula from a microburette. The segments were tied off, the abdomen sewed up and the animal left in warm surroundings for the period of absorption. After this interval the animal was opened up again and killed by making a snip in the heart. Each loop was carefu: :ly removed from the abdomen, care being taken when removing the mesentery to avoid stretching the loops. The loops were measured by placing them vertically against a fixed centimetre scale.

Fig. 11.

Fig. 11 shows the position of the ligatures on the gut of an anaesthetised rat during a routine absorption experiment to determine the rates of absorption of amino acids from upper and lower loops respectively.

Ligature tied at once.

Ligature left loose initially.



Thoracic Viscera and Liver Removed to show Alimentary Tract.

after Greene, 1935.

Figure 11.

Small openings were made in each loop just below the upper and lower ligatures. A cannula was introduced into the upper opening, and the contents of the gut washed into a medium-sized funnel draining into a 600 c.c.beaker. The loops were then slit open and the mucosa thoroughly washed with warm saline solution (1% w/v). The total volume of the contents of the loops and the solution used to wash them amounted to about 200 c.c. volume in each case.

Estimation of recovered materials

To these solutions were added 10 c.c. of 3% w/v) acetic acid solution which produced an acidity optimum for heat coagulation and flocculation of the proteins (Wilson and Lewis, 1929). The solutions were heated to boiling to destroy any proteolytic enzymes present and coagulate the proteins, concentrated to about 50 c.c., allowed to stand until cool, and filtered. To each of the filtrates were added 5 c.c. of a 10% (w/v) solution of sodium tungstate, 0.67N Sulphuric acid sufficient to cause the appearance of a flocculent precipitate, and distilled water to give a final volume of 100 c.c. The solutions were placed in an ice chamber where they remained overnight. The precipitates were then removed by filtration. To 20 c.c. of each of the filtrates, a few drops of a 10% (w/v) solution of

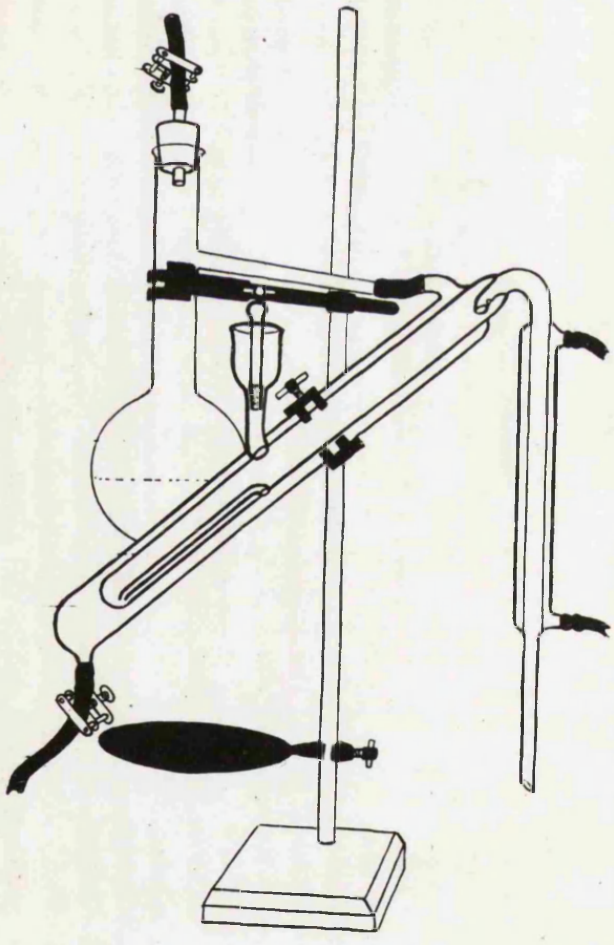
sodium hydroxide were added until the reaction was weakly acid to litmus, and the solutions gently brought to the boil. They were then made slightly alkaline by further addition of the sodium hydroxide solution and boiled for five or six minutes to remove any ammonia present. The solutions were made acid with a 3% (w/v) solution of acetic acid, concentrated, and made up to a final volume of 25 c.c. The amino acid nitrogen content of the solutions was found by micro Kjeldahl analysis using Markham's (1942) steam distillation apparatus, (Fig.12), and the amino acid recovery estimated. As a control 1 c.c. of the original amino acid solution was diluted to 10 c.c. and the amino acid nitrogen content estimated.

Accuracy of method:

It was necessary to demonstrate that quantitative recovery of added amino acid nitrogen could be obtained by the above procedure. 1.5 c.c. of an amino acid solution were run into a ligatured loop of the small intestine of an anaesthetised rat and the loop immediately excised and washed. In this way 96% of the original amino acid could be recovered. This percentage recovery could be obtained only by slitting open the excised loop and washing the mucosa thoroughly with warm saline solution, otherwise only 84% of the original amino acid could be recovered. Using the same operative procedure

Fig. 12.

Fig. 12 shows a diagram of the steam distillation apparatus with steam generator and connections suitable for micro Kjeldahl analysis. The apparatus has a minimum of rubber connections and is blown from "Phoenix" resistance glass.



The steam-distillation apparatus with steam generator and connexions.

after Markham, 1942.

Figure 12.

and determining the amino acid nitrogen by the gasometric method of Hiller and Van Slyke (1922). Schofield and Lewis (1947) obtained a recovery of 93 - 94%.

Other methods of estimation:

Rappaport and Eichorn's (1947a,b) rapid method of determining non protein nitrogen was tried in order to eliminate the lengthy period of time (4½ hours) necessary for Kjeldahl digestion. This method had to be abandoned in favour of the micro Kjeldahl technique owing to the large experimental error involved.

Bacteria of the gut:

Estimations were made to determine if the bacteria of the gut utilised the amino acids presented to them. A given volume of an amino acid solution of known strength was incubated at 37°C for 1 hour with a 1% (w/v) saline solution which had been perfused through the lumen of the small intestine of an anaesthetised rat. After incubation, the mean percentage of the amino acid in the solution was found to be 96%, showing that during the period of absorption the bacteria of the gut do not use the amino acid to any detectable extent for synthetic or other purposes.

Decerebration:

An attempt was made to measure the rate of

absorption of amino acids from the gut of decerebrate rats. It was thought that the use of an anaesthetic might influence the rate of absorption of the amino acids under investigation. The method employed was a modification of that used by Landis (1930) and Bell, Horne, and Magee (1933). For 24 hours previous to the experiment the rats were allowed water only. Decerebration was carried out on 24 animals. Rats weighing about 350-450 gms. were lightly etherised, the carotid arteries ligatured and the trachea cannulated. The skin of the head was incised and the skull bared. Trephine openings were made in the cranium and the cerebral hemispheres carefully scooped out. The loss of blood was considerable; it occurred largely when trephine holes were bored. Manual artificial respiration was often necessary at this stage. Spontaneous movements were, in the main, absent. Of the animals prepared in this way only two survived the absorption period. The majority died during or after decerebration, and the remainder long before the absorption period was completed. The cause of death appeared to be haemorrhage. Bell, Horne, and Magee (1933) assumed the cause of death in their decerebrate rat preparations might be compression of the medulla due to blood clots. Their operative procedure differed from the above method in that the brain stem was sectioned but the cerebral hemispheres were not

scooped out. Various methods of decerebration including those of Bell, Horne, and Magee (1933) and Schmidt (1923) were tried without success. Further attempts to decerebrate rats were therefore abandoned owing to the exceedingly low survival rate.

Preparation of solutions of amino acids:

The amino acids used were glycine, L(+) alanine, $[\alpha]_D = +14.70^\circ$ (0.97N HCl), L (-) phenylalanine $[\alpha]_D = -35.14^\circ$ (water), and D(-) isoleucine $[\alpha]_D = -10.50^\circ$ (water) supplied by Roche Products, Ltd. Solutions, isosmotic and half isosmotic with the blood, were made up 24 hours before use. The amount of amino acid in each case required to make an isosmotic solution was calculated from a given value for the depression of freezing point of blood. The osmotic pressure of blood depends on its molecular concentration and can be determined, e.g., by determination of the freezing point. The depression of freezing point ($\triangle$) of mammalian blood in equilibrium with air is about 0.53°C. This is equal to that of a 0.9% solution of sodium chloride and is therefore often taken as isosmotic with the blood. The average osmotic pressure of blood in the adult male is equal to that of a 0.945% solution of sodium chloride ($\triangle$ = 0.553°C) when the blood is in equilibrium with 5% CO_2. Actually the osmotic pressure of the contents of the red

corpuscles is a little lower than that of the plasma but the difference is not enough for haemolysis appreciably to alter the freezing point or the osmotic pressure of the blood.

The depression of freezing point ($\triangle$) of a given solvent is proportional to the molecular concentration of the solute; and therefore equimolecular solutions have the same freezing point.

$$\triangle = \frac{K\,n}{W}$$

where n is the number of gramme molecules solute dissolved in W grammes solvent. K is a constant and is equal to $\frac{RT^2}{l}$

$$\triangle = \frac{RT^2}{l} \cdot \frac{n}{W}$$

where l is the latent heat of fusion of the crystalline solvent, and T the absolute temperature of the freezing point. In the case of water, therefore,

$$\triangle = \frac{1.988 \times 273^2}{79.77} \cdot \frac{n}{W} = \frac{1858 \cdot n}{W}$$

The depression of freezing point is proportional to the relative lowering of the vapour pressure and consequently it is also proportional to the osmotic pressure of the solution.

Therefore $\triangle = \frac{1858.n}{W} = \frac{1858.w}{m.W}$

where m is the molecular weight of the solute in the solution and w and W are the weights of solute and solvent respectively. Assuming $\triangle$ to be 0.53°C, it was a simple matter to calculate w, the weight of amino acid in 100 c.c. of water which would provide a solution isosmotic with blood.

Histology:

An examination of the histological picture of the epithelium of the small intestine was made. It was thought advisable to see if there were any signs of gross injury to the epithelial membrane. At the end of each experiment a small piece of the intestinal loop was fixed in Bouin's fluid and embedded in wax. It was cut at 8 μ and stained with Haematoxylin and Orange G. Microscopical examination revealed no sign of epithelial desquamation in the case of isosmotic glycine and half isosmotic solutions of glycine, alanine, isoleucine and phenylalanine (Fig. 13).

b) RESULTS:

Presentation of tables:

The results of each experiment were recorded in a protocol as in Table 2.

Fig. 13.

Fig. 13 shows the histological appearance of the upper loop of the small intestine of the rat after half isosmotic glycine had been left in the lumen of the gut for 1 hour. The epithelium is absolutely intact. In order to obtain such a picture it is necessary to remove the gut from a living anaesthetised rat and to place the tissue at once in fixative. X 126.

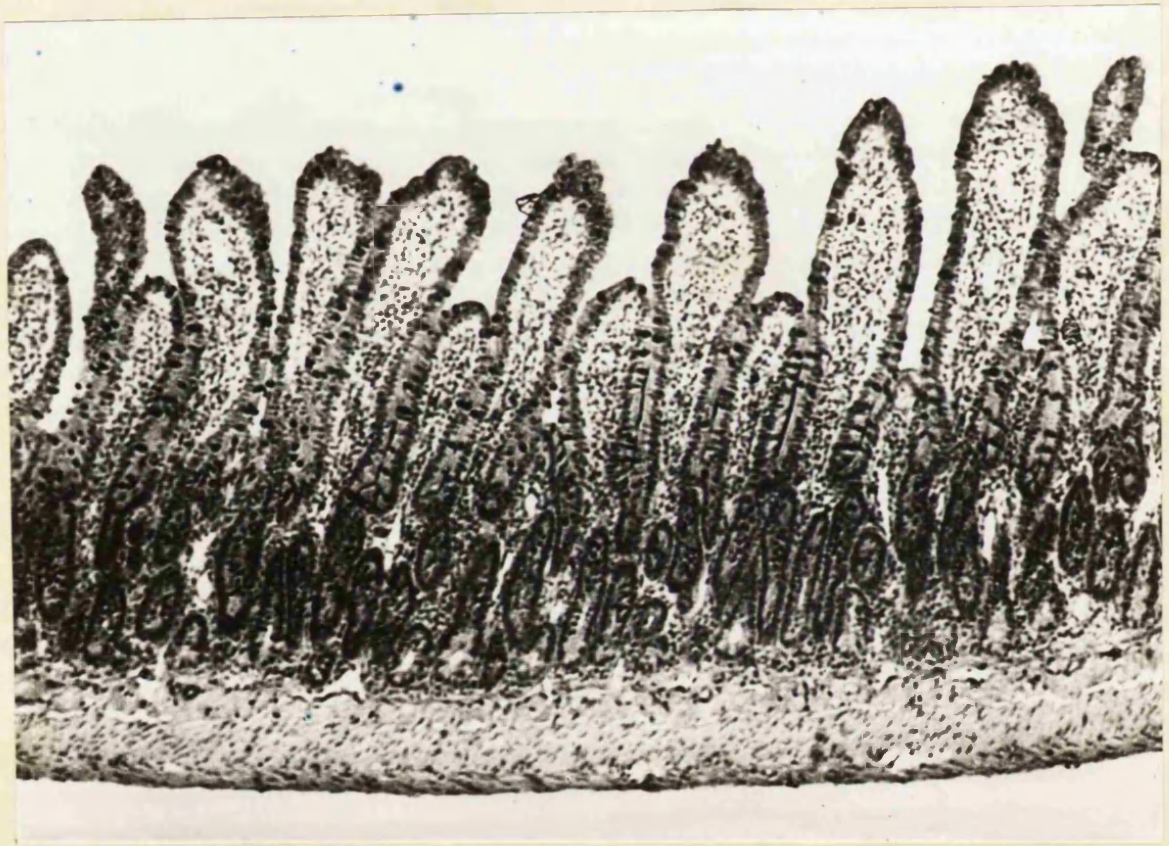


Figure 13.

In Tables 3, 4, 5, 6 and 7 all the data necessary for the determination of the relative absorption values in upper and lower loops of the small intestine of the rat are recorded. Table 3 records the results obtained with isosmotic glycine in 4 rats. In all of these the time of absorption was 90 minutes. Table 4 shows the results obtained with half isosmotic glycine in 8 rats. In all of these the time of absorption was 60 minutes.

Table 5 gives the results for the absorption of half isosmotic L (+) alanine in 10 rats. In seven of these the time of absorption was 60 minutes, and in the other three, 40 minutes. Similarly, Tables 6 and 7 show the absorption values for half isosmotic L(-)phenylalanine and half isosmotic D(-)isoleucine respectively. In all of these the time of absorption was 60 minutes. The number of experimental animals in each case was 7. Results from all experiments not wholly free from technical fault are omitted.

Volume of original solution placed in gut:

It will be noticed in the column recording the volume of glycine solution placed in each loop that in early experiments 1.5 c.c. of glycine per loop were

used whereas in later experiments the amount of solution placed in each loop is increased to 2.0 c.c. It was decided to increase the volume of solution from 1.5 c.c. in the case of the isosmotic solution to 2 c.c. in the case of the half isosmotic solution. The intestinal mucosa was thereby presented with more molecules of amino acid from the 2 c.c. of solution. Moreover, the percentage recovery was higher thus making the subsequent estimation of amino nitrogen easier.

The increase in volume from 1.5 to 2 c.c. of amino acid solution introduced did not bring about undue distension in the gut loops.

Period of absorption: The time allowed for absorption is obviously a factor of no little moment. Ideally, the duration should be such that the animal has full opportunity to recover from the disturbances due to laparotomy and insertion of the amino acid solution. In addition the time should be of such length that absorption is fully under way but should not be so long that practically all the amino acid is absorbed. For example, choice of too long an absorption period may give the impression that two amino acids have equal absorption rates if the time is sufficient to allow practically 100% of the more slowly absorbed amino acid to disappear from the gut lumen. From the technical point

TABLE 2.

Typical protocol used in recording results.

Rat serial number:- 16 (white/black; Rowett) Date:-23/7/48.

Sex: Male. Weight:- 215 g.

Pre-experimental treatment:- Water only for 24 hours.

Anaesthetic:- Urethane. Dose:- 1.6 mg. per g. rat.

Substance tested:- Glycine.

Concentration:- Half iso-osmotic. (1.0804 g/100 c.c.)

Volume:- 2.0 c.c. per loop.

Time of absorption:- 60 minutes.

Region of gut:- Upper loop of small intestine length 18 cm.
Lower loop of small intestine length 22 cm.

Region.	Initial.	Final.	Difference.	mg.absorbed/cm.gut
Upper loop.	21.79	7.48	14.31	0.80
Lower loop.	21.79	8.88	12.91	0.59

Comments:- Nil c.c. additional urethane given.

Condition of animal:- Good.

-------oOo-------

of view, moreover, estimation of minute quantities of amino acids is undesirable.

An absorption period of 60 minutes was chosen for all half isosmotic amino acids used and 90 minutes for isosmotic glycine. Such an interval allows fully half the amino acid to disappear from the gut lumen. Cori (1926-27) and later workers who applied his technique, chose absorption periods ranging from 1 hour to 4 hours for all amino acids, but the concentrations of amino acids introduced into the gastrointe-stinal tract were much greater than isosmotic. Cori fed a 15% solution of amino acid by stomach tube to rats.

Column headings:

The column under the heading "actual absorption" represents the amount of amino acid which had disappeared from the gut lumen. This amount is also expressed as a percentage. To obtain the absorption rate per unit length of gut the actual absorption in mg. from each loop is divided by the length of the loop in cms. The absorption rate per sq. cm. gut is obtained by dividing the actual absorption by the mucosal area. Wood (1944) has provided mean figures for mucosal area in a comparison between surface area of the mucosa in jejunum, ileum and colon per cm. length of gut. These figures are, in the case of the rat:-

Jejunum. 8.5 sq.cm./ cm. serosal length.

Ileum. 5.I sq.cm./ cm. serosal length.

The column under the heading actual absorption/molecular weight gives the milli molar absorption rate of the amino acids. In Table 8 the average absorption values, the actual absorption, the rate of absorption per unit length, the rate of absorption per unit surface area, and the milli molar absorption rate, over a period of 60 minutes in the case of half isosmotic amino acids are recorded for upper and lower loops of the small intestine in the rat. The average absorp: :tion values for isosmotic glycine, where the time of abser: :ption is 90 minutes, are given "corrected" for an absorption time of 60 minutes.

Graphs: In Fig. I4, rates of absorption in mg/hour of glycine, L(+)alanine, L(-)phenylalanine and D(-) isoleucine from upper and lower loops of the small intestine of anaesthetised rats are plotted against molecular weight. Similarly, in Fig. I5, the rates of absorption of glycine, L(+) alanine, L(-)phenylalanine and D(-)isoleucine expressed in mg/ hour from upper and lower loops of the small intestine of anaesthetised rats is plotted against the apparent molal vol: :ume of these amino acids.

In Figs. I6 and I7 the millimolar absorption rates of glycine, L(+)alanine, L(-)phenylalanine and D(-)isoleucine from upper and lower loops of the small intestine of anaesthetised rats are plotted against molecular weight and against the

apparent molal volume of these amino acids.

The values of the apparent molal volume used were taken from the reports of Dalton and Schmidt (1933; 1935), Cohn et al (1934; 1936) and Cohn and Edsall (1943), their observed values being used when given. When these were not available, the values were calculated according to their method. The equation from which the apparent molal volume was calculated was:-

$$\Phi(V_2) = \frac{1000}{C}\left(\frac{d_1 - d}{d_1}\right) + \frac{M_2}{d_1}$$

where C is the concentration in moles per litre and d and d_1 are the density of the solution and the density of pure water. Another method by which the apparent molal volume may be calculated is that of Traube (1899), using the molal volume increments for certain atoms and groups at 15°C:-

	C	H	N	O'	O''
Volume of atoms, c.c/mole.	9.9	3.1	1.5	2.3*	5.5

	$-NH_2$	$-CH_2$	-COOH	-CONH-
Volume of groups, c.c/mole.	7.7	16.1	15.9	20.0

(* Traube believed that a second hydroxyl group, or the second oxygen in a carboxyl group occupied less space than the first). The effect of temperature on these apparent molal volumes is relatively slight.

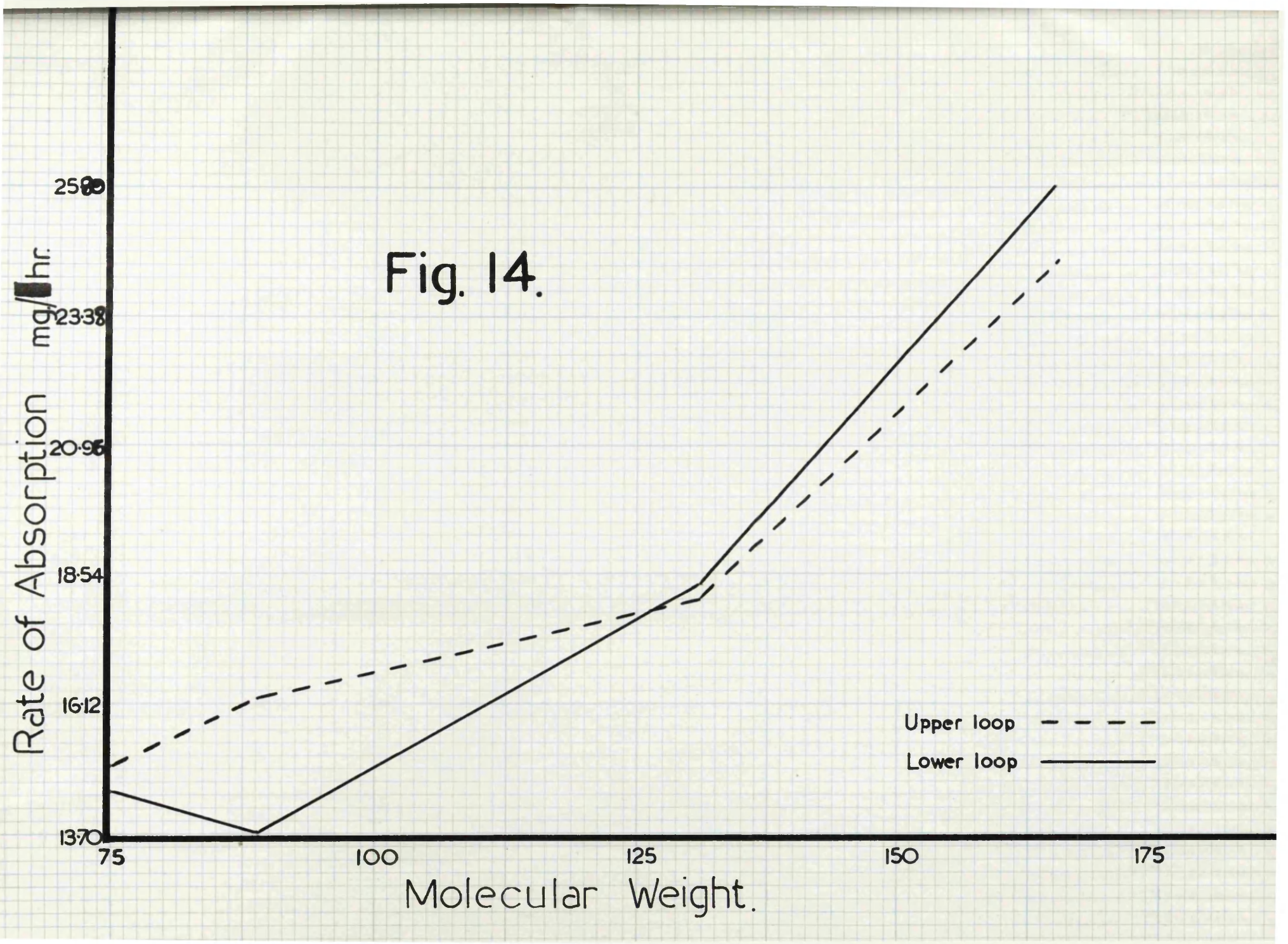


Fig. 14.
Rate of Absorption mg/ hr.
25·80
23·38
20·96
18·54
16·12
13·70
75
100
125
150
175
Molecular Weight.
Upper loop
Lower loop

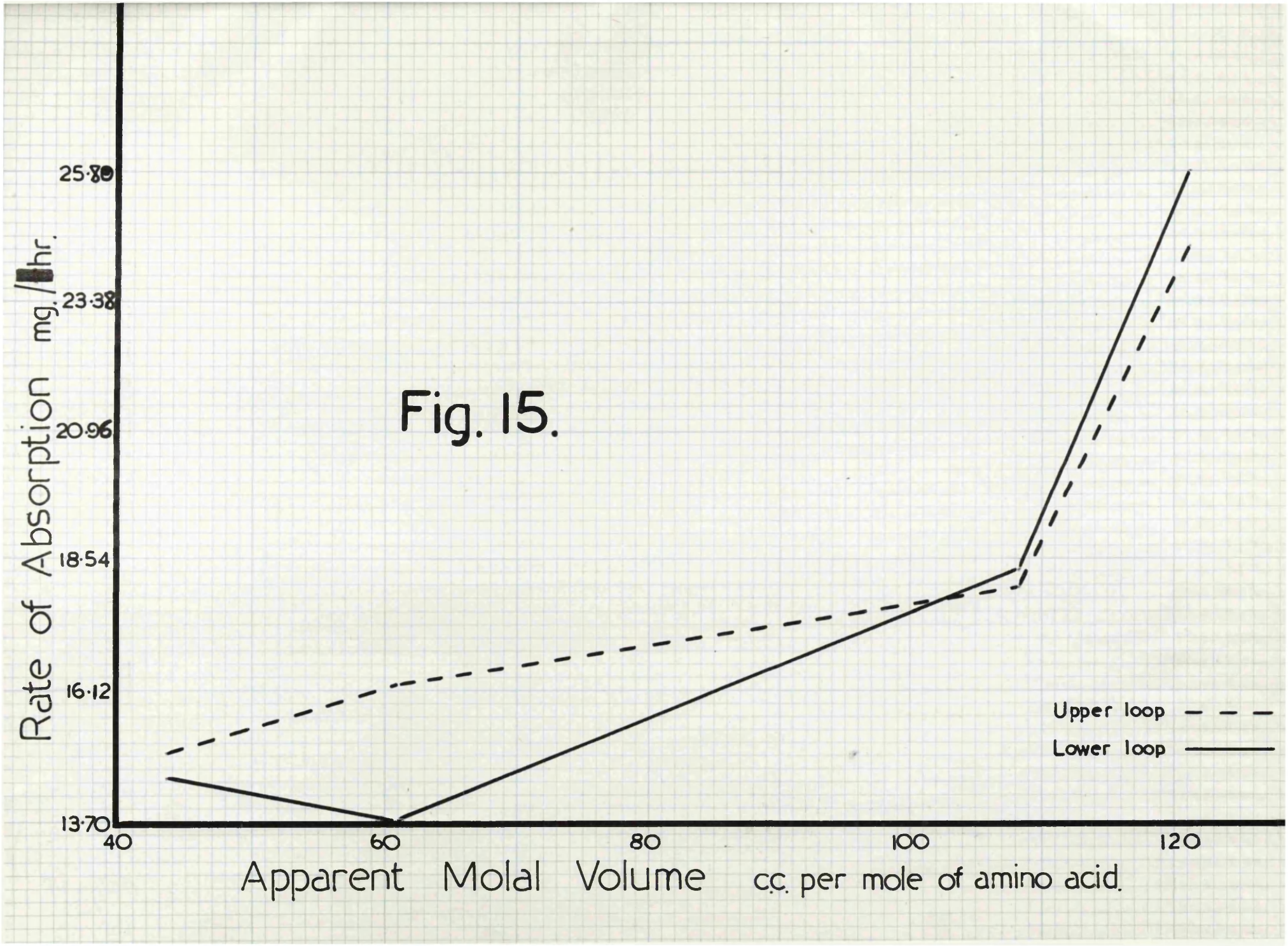


Fig. 15.
Rate of Absorption mg./hr.
25·80
23·38
20·96
18·54
16·12
13·70
40
60
80
100
120
Apparent Molal Volume c.c. per mole of amino acid.
Upper loop
Lower loop

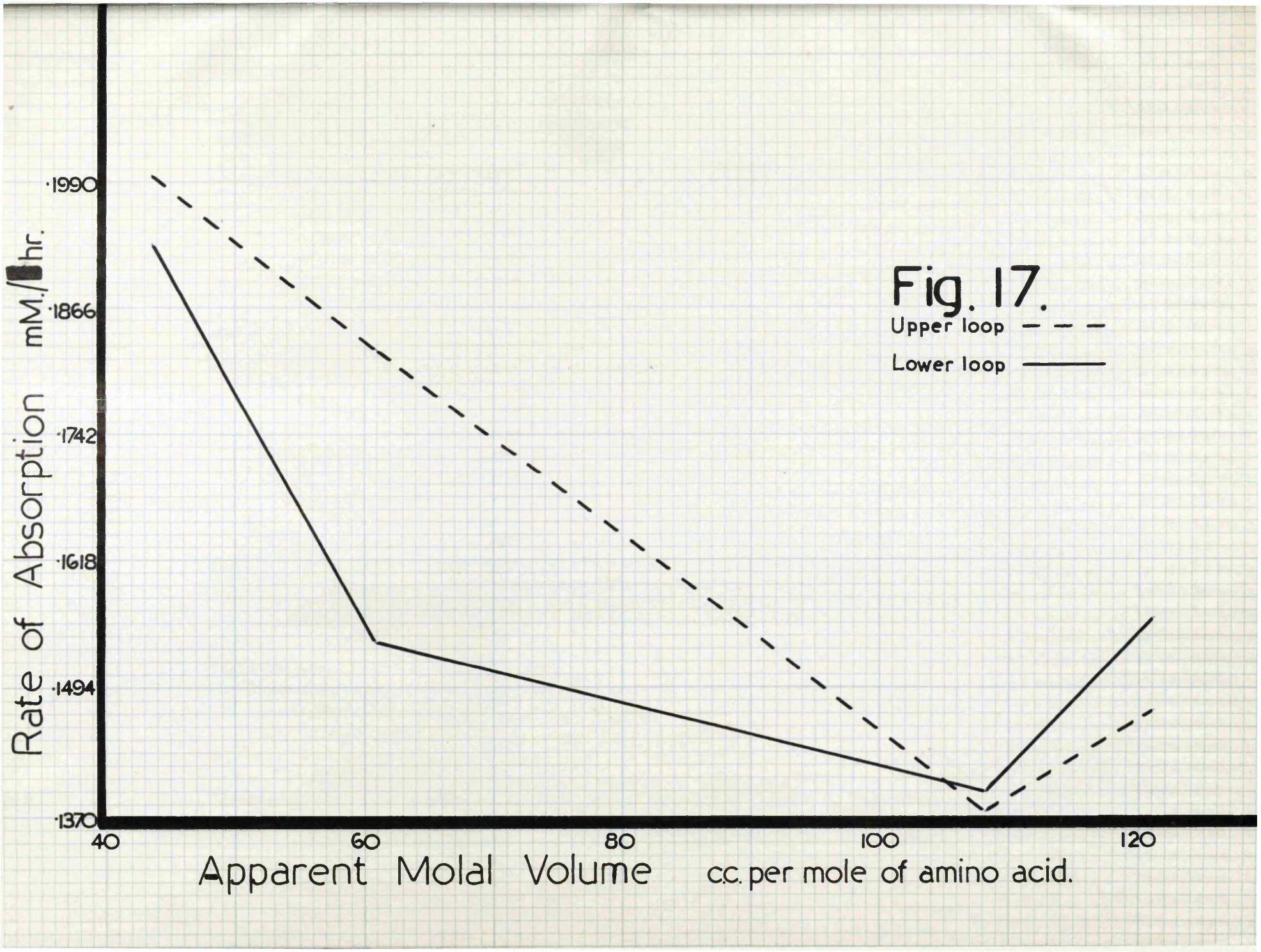

Rate of Absorption mM./hr.
·1990
·1866
·1742
·1618
·1494
·1370
40
60
80
100
120
Apparent Molal Volume c.c. per mole of amino acid.
Upper loop
Lower loop

Fig. 17.

The apparent molal volume of a compound, however, is always greater than the sum of the volumes assigned to its constituent atoms or groups. This additional volume - termed the "Co-volume" by Traube - varies very little from molecule to molecule and is approximately 13 c.c/g.mole.

Statistical Analysis of Results:

The actual absorption values of glycine, L alanine, L-phenylalanine and D-isoleucine in upper and lower loops of the small intestine of the rat given in Tables 3, 4, 5, 6 and 7, are compared by the statistical methods described by Chambers (1946).

In each Table the absorption values in the upper loop are compared with those in the lower loop, and the significance of the difference between the means is stated.

Notation: A certain amount of symbolism is essential in the description of statistical methods. If X is a variable which has different values, X_1, X_2, X_3, etc., then the arithmetic mean of a number N of such values is the sum of the various values of X, which is denoted by S(X), divided by N, the number of them. In general, therefore,

$$\bar{X} = \frac{S(X)}{N}$$

hence $\bar{X}$ denotes "the mean of X". In the tables, $\bar{X}_1$ denotes the mean of the actual absorption values found in the upper loop and $\bar{X}_2$ the mean of the actual absorption

TABLE 3

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP Initial vol. per loop cc.	UPPER LOOP Length of gut. cm.	UPPER LOOP Absorption Actual mg	UPPER LOOP Absorption %	UPPER LOOP Absorption Per cm. gut mg.	UPPER LOOP Absorption Per sq. cm. gut. mg.	UPPER LOOP Absorption Actual / Mol. Wt.	LOWER LOOP Initial vol. per loop cc.	LOWER LOOP Length of gut. cm.	LOWER LOOP Absorption Actual mg.	LOWER LOOP Absorption %	LOWER LOOP Absorption Per cm. gut. mg.	LOWER LOOP Absorption Per sq. cm. gut. mg.	LOWER LOOP Absorption Actual / Mol. Wt.
10/4/47	Rat	M	355	90	1.5	30.0	19	59	.64	.08	.26	1.5	27.0	14	43	.52	.10	.19
14/4/47	Rat	M	415	90	1.5	30.0	20	60	.66	.08	.26	1.5	28.0	17	53	.62	.12	.23
16/4/47	Rat	M	365	90	1.5	30.0	24	72	.78	.09	.31	1.5	30.0	17	52	.56	.11	.23
18/6/47	Rat	M	360	90	1.5	30.0	24	70	.81	.10	.33	1.5	30.0	17	51	.55	.11	.22

GLYCINE.

TABLE 4

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: Actual %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: Actual %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
27/7/48	Rat	M	175	60	2.0	28.0	16	75	.58	.07	.22	2.0	29.0	12	55	.41	.08	.16
28/7/48	Rat	M	205	60	2.0	29.0	14	66	.49	.06	.19	2.0	25.0	18	81	.71	.14	.23
30/7/48	Rat	M	185	60	2.0	24.0	16	74	.67	.08	.22	2.0	29.0	16	74	.56	.11	.22
3/8/48	Rat	M	165	60	2.0	20.0	14	65	.70	.08	.19	2.0	29.0	12	55	.42	.08	.16
4/8/48	Rat	M	215	60	2.0	18.0	14	66	.80	.09	.19	2.0	22.0	13	59	.59	.12	.17
5/8/48	Rat	M	224	60	2.0	25.0	15	68	.59	.07	.20	2.0	25.0	14	67	.56	.06	.19
6/8/48	Rat	M	186	60	2.0	20.0	13	59	.67	.08	.18	2.0	25.0	18	81	.70	.14	.23
7/8/48	Rat	M	183	60	2.0	29.0	16	74	.56	.11	.21	2.0	24.0	13	58	.53	.10	.17

GLYCINE

TABLE 5

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual / Mol.Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual / Mol.Wt.
28/8/48	Rat	M	220	60	2.0	26.0	15	57	.56	.07	.16	2.0	23.0	15	58	.64	.13	.16
29/8/48	Rat	M	232	60	2.0	22.0	16	64	.78	.09	.18	2.0	30.0	16	62	.52	.10	.18
30/8/48	Rat	M	153	60	2.0	31.0	13	49	.40	.05	.14	2.0	26.0	14	54	.54	.10	.15
5/9/48	Rat	M	201	60	2.0	29.0	22	87	.76	.09	.52	2.0	27.0	11	41	.39	.08	.12
6/9/48	Rat	M	183	60	2.0	25.0	15	58	.59	.07	.17	2.0	26.0	15	57	.56	.11	.16
7/9/48	Rat	M	200	60	2.0	30.0	17	68	.58	.07	.19	2.0	30.0	16	62	.52	.10	.18
8/9/48	Rat	M	205	60	2.0	27.0	16	64	.60	.07	.18	2.0	22.0	11	43	.50	.10	.12
12/9/48	Rat	M	244	40	2.0	27.0	15	57	.54	.06	.16	2.0	32.0	11	43	.34	.07	.12
13/9/48	Rat	M	231	40	2.0	28.0	17	66	.67	.08	.19	2.0	30.0	13	51	.43	.09	.15
14/9/48	Rat	M	195	40	2.0	20.0	10	40	.50	.06	.11	2.0	18.0	18	71	1.00	.20	.20

ALANINE.

TABLE 6

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
16/9/48	Rat	M	230	60	2.0	31.0	17	35	.54	.06	.10	2.0	30.0	25	53	.83	.16	.15
17/9/48	Rat	M	225	60	2.0	25.0	25	54	1.01	.12	.15	2.0	23.0	21	45	.92	.18	.13
20/9/48	Rat	M	207	60	2.0	29.0	28	60	.98	.12	.17	2.0	27.0	23	49	.86	.17	.14
21/9/48	Rat	M	225	60	2.0	27.0	27	56	.98	.12	.16	2.0	25.0	24	50	.95	.19	.14
24/9/48	Rat	M	195	60	2.0	28.0	28	59	.98	.12	.17	2.0	31.0	29	61	.93	.18	.17
25/9/48	Rat	M	175	60	2.0	23.0	23	48	.99	.12	.14	2.0	30.0	28	60	.94	.18	.17
26/9/48	Rat	M	225	60	2.0	23.0	24	51	1.03	.12	.14	2.0	21.0	31	65	1.46	.29	.19

PHENYLALANINE

TABLE 7

					UPPER LOOP							LOWER LOOP						
							Absorption							Absorption				
Date	Animal	Sex	Weight gm.	Time of absorption min.	Initial vol. per loop cc.	Length of gut cm.	Actual mg	%	Per cm. gut mg.	Per sq. cm. gut. mg.	Actual / Mol. Wt.	Initial vol. per loop cc.	Length of gut. cm.	Actual mg.	%	Per cm. gut. mg.	Per sq. cm. gut. mg.	Actual / Mol. Wt.
1/10/48	Rat	M	218	60	2.0	35.0	18	48	.51	.06	.14	2.0	26.0	18	49	.70	.14	.14
4/10/48	Rat	M	176	60	2.0	26.0	17	48	.66	.08	.13	2.0	29.0	21	58	.73	.14	.16
5/10/48	Rat	M	210	60	2.0	25.0	20	53	.80	.09	.15	2.0	27.0	17	46	.64	.12	.13
6/10/48	Rat	M	164	60	2.0	22.0	19	50	.84	.10	.14	2.0	22.0	17	46	.78	.15	.13
7/10/48	Rat	M	167	60	2.0	23.0	16	41	.67	.08	.12	2.0	26.0	21	55	.80	.16	.16
8/10/48	Rat	M	193	60	2.0	22.0	17	46	.79	.09	.13	2.0	23.5	19	51	.80	.16	.14
11/10/48	Rat	M	175	60	2.0	28.0	20	53	.71	.08	.15	2.0	30.0	15	39	.48	.10	.11

I S O L E U C I N E

TABLE 8.

ABSORPTION.
Mean Values.

Amino acid used.	Time of Absorption.	UPPER LOOP. Initial vol. per loop.	Actual.	%	per cm. gut.	per sq. cm. gut.	Actual/M.W.	LOWER LOOP. Initial vol. per loop.	Actual.	%	per cm. gut.	per sq. cm. gut.	Actual/M.W.	
	mins.	c.c.	mg.		mg.	mg.		c.c.	mg.		mg.	mg.		
Isosmotic GLYCINE.	60	1.5	14.5	65.2	.48	.06	.19	1.5	10.8	50.0	.38	.07	.14	4 Results.
Half isosmotic GLYCINE.	60	2.0	15.0	68.3	.63	.07	.20	2.0	14.5	66.4	.56	.11	.19	8 Results.
Half isosmotic ALANINE.	60	2.0	16.3	64.0	.61	.07	.18	2.0	13.7	53.8	.52	.10	.15	7 Results.
Half isosmotic PHENYLANALINE.	60	2.0	24.4	51.9	.93	.11	.15	2.0	25.8	54.8	.98	.19	.16	7 Results.
Half isosmotic ISOLEUCINE.	60	2.0	18.0	48.5	.71	.08	.14	2.0	18.3	49.0	.70	.14	.14	7 Results.

(Results from all experiments not wholly free from technical fault are omitted).

Actual time of absorption - 90 mins., results" corrected" to 60 mins.

values found in the lower loop.

Vertical lines drawn each side of a quantity denote "the positive numerical value of", e.g. $|\bar{X}_1 - \bar{X}_2|$ denotes "the positive numerical value of the difference between the means $\bar{X}_1$ and $\bar{X}_2$." Using this notation it does not matter whether $|\bar{X}_1 - \bar{X}_2|$ is written or $|\bar{X}_2 - \bar{X}_1|$.

The ratio of the difference between the means to the standard error of the difference is denoted by t. The standard error of the difference is the ratio of the standard deviation, σ, of the sample to the square root of N, the number of observations in it. The standard deviation, σ, is the square root of the mean of the squares of the deviation of the observations from their arithmetic mean:— $\sigma = \sqrt{\frac{S(X - \bar{X})^2}{N}}$. However, the number of observations in each of Tables 4, 5, 6 and 7 is less than 50 and the use of the formula $t = \frac{\text{difference between the means}}{\text{standard error of the difference}}$, is not a sufficiently strict test. Corrections, given by Chambers (1946) are made to allow for the small number of observations in each group.

P denotes the "probability" of an event and may be defined as the expected frequency of occurrence of this event among events of a like sort. Fisher and Yates (1943) have tabled the distribution of t corresponding

to different values of n ($n = N_1 + N_2 - 2$ in this case) and probabilities ranging from 0.9 to 0.001. Where the probability, P, is 0.05 say, the odds against values of t as big as or bigger than these occurring by chance are 19:1 i.e. the probability of their occurring by chance is 0.05. If the calculated value of t is more than that given in the table for the appropriate value of n then the difference between the means is said to be significant, that the difference is not likely to have arisen by chance. The statistical values obtained for $\bar{X}_1$, $\bar{X}_2$, $|\bar{X}_1 - \bar{X}_2|$ t and P are tabulated in Table 9.

The percentage absorption values of glycine, L(+) alanine, L(+)phenylalanine and D(-)isoleucine in upper and lower loops of the small intestine of the rat given in Tables 3, 4, 5, 6 and 7 are also statistically compared. In each Table the percentage absorption values in the upper loop are compared with those in the lower loop. The statistical values obtained are tabulated in Table 10.

Notation: Values for the difference between the percentage absorption in the upper loop and that in the lower loop are called X, and their sum S(X). $\bar{X}$ is obtained by dividing S(X) by N, the number of observations t is calculated from Chamber's formula $t = \bar{X} \div \frac{\sigma}{N}$

TABLE 9.

Table.	$\bar{X}_1$	$\bar{X}_2$	$\lvert\bar{X}_1-\bar{X}_2\rvert$	t	P	Difference between the means.
3.	21.7	16.2	5.5	0.36	0.8-0.7	Insignificant.
4.	14.9	14.4	0.5	0.51	0.7-0.6	Insignificant.
5a.	16.3	13.7	2.6	1.83	0.1-0.05	Insignificant.
5b.	13.8	14.1	0.3	0.21	0.9-0.8	Insignificant.
6.	24.4	25.8	1.4	0.10	0.9	Insignificant.
7.	18.0	18.3	0.3	0.33	0.8-0.7	Insignificant.

TABLE 10.

Table	$\bar{X}$	$S(X-\bar{X})^2$	t	P	Difference between the means and zero.
3.	15.60	97.44	5.46	0.02-0.01	Significant.
4.	2.00	1440.92	0.39	0.80-0.70	Insignificant.
5.	1.13	2436.88	0.15	0.90-0.80	Insignificant.
6.	-2.90	840.60	-0.60	0.60-0.50	Insignificant.
7.	0.50	568.90	0.14	0.90-0.80	Insignificant.

TABLE 11.

Loops.	σ_x	σ_y	$\frac{S(XY)}{N}$	$\bar{XY}$	r	P	
Upper Loop.	3.6	34.1	1675.5	1563.5	.906	0.1-0.05	Barely Significant
Lower loop.	4.8	34.1	1683.8	1534.7	.912	0.1-0.05	Barely Significant

Correlation:

It is desirable to know to what extent one variable, in this case the actual rate of absorption is related to another, the apparent molal volume. When the two variables are numerical and normally distributed, the association, or correlation, between them may best be measured by a method known as the product-moment method.

The product-moment coefficient of correlation, r, is that ratio which expresses the extent to which changes in one variable are accompanied by, or are dependent upon, changes in a second variable.

Correlation is rarely computed when the number of cases is less than 25, so that the examples here presented must be considered to have illustrative value only.

Notation: The values of X, the actual rates of absorption from the Table of averages (Table No. 8) and Y, the apparent molal volumes of the amino acids studied are set down in two parallel columns so that the pair of readings in each horizontal row belongs to the same amino acid. Two more columns are derived, headed X^2 and Y^2, by squaring the terms in the first two columns. The totals of these columns are given by $S(X)$, $S(Y)$, $S(X^2)$ and $S(Y^2)$, and these data permit calculation of the means

and standard deviations of X and Y by the formulae:

$$\bar{X} = \frac{S(X)}{N}, \quad \bar{Y} = \frac{S(Y)}{N}, \quad \sigma_x = \sqrt{\frac{S(x^2)}{N} - \bar{x}^2} \quad \sigma_y = \sqrt{\frac{S(Y^2)}{N} - \bar{Y}^2}$$

A fifth column, headed XY, is obtained by multiplying togeth: :er corresponding X's and Y's in the first two columns. The total of this column is S(XY).

The coefficient of correlation, r, is obtained from the formula

$$r = \frac{\frac{S(XY)}{N} - \bar{X}\bar{Y}}{\sigma_x \cdot \sigma_y}$$

If there is complete positive correlation (perfect relationship) between X and Y, r has the value of + 1; if there is complete negative correlation (negative or inverse relationship is perfect) it has the value of -1, and incomplete correlation gives decimal values for r between + 1 and -1. When there is no relation at all between the variables r = 0.

The correlation between the actual absorption values of the amino acids glycine, L(+) alanine, L(-) phenylalanine and D(-) isoleucine in upper and lower loops of the small intestine of the unaesthetised rat, given in Table of averages (Table 8) and their apparent molal volumes is measured by the product moment method described by Chambers (1946).

The values obtained for σ_x, σ_y, $\frac{S(XY)}{N}$, $\bar{x}.\bar{y}$, r and P are tabulated in Table 11.

The significance of r when N = 50 or less is found from Fisher and Yates (1943) Tables. In using this table n = N - 2 where N is the number of pairs of observations in the correlation. If the calculated value or r is as big or bigger than the value given in the table for the appropriate value of n, the correlation differs significantly from zero, i.e. it indicates a real degree of association between the two variables.

Statistical comparison of the actual absorption values in Tables 3, 4, 5, 6 and 7 indicates no difference between the rates of absorption (expressed as mg. or percentage) of the amino acids in upper and lower loops of the small intestine of the anaesthetised rat.

c) Discussion:

Methods of expressing rates of absorption.

a Unit mucosal surface area:

Wood's figures (1944) for the relative mucosal area in jejunum and ileum were used to find the rates of absorption of glycine, L(+) alanine, L(-)phenylalanine and D(-)isoleucine per sq. cm. mucosa in upper and lower loops of the small intestine. These results are seen in

Tables 3, 4, 5, 6 and 7, and brought together in Table 8. This is the ideal method of expressing rates of absorption since the mucosal area across which the diffusible substances pass is of fundamental importance. Workers in this field have always attempted to standardise and compare their results by expressing the absorption rate of substances from the gut lumen in terms of quantity absorbed per unit gut length, or body weight, or body surface area in unit time. These measurements have been used on the assumption that gut length, gut weight, body weight and body surface area were functions of mucosal surface area of the gut. So far as I know, no one has expressed the rates of absorption of amino acids in the rat in terms of quantity absorbed per unit mucosal surface area. All methods of expressing rates of absorption without reference to the mucosal area suffer from one or more defects.

b Unit length:

The measurement of the gut length involves obvious inaccuracies which cannot be avoided in view of the tortuous course followed by the gut, and the attachment of the gut to the mesentery. Killing the animal, freeing the gut from the mesentery and suspending it against a vertical measure gives no true indication of the length of the gut before death. Evidence is accumulating that in life the entire gut is much shorter

than it is post-mortem (Papé and Cannon, 1932; 1940).

c. Unit weight:

Theoretically, gut weight ought to be a more reliable measurement than gut length. Presumably the weight of the gut will be directly proportional to the number of villi and therefore to the mucosal area of the gut if the other layers of the gut wall are also directly proportional to the weight of the gut. Unfortunately, the moisture content of each loop of gut varies as it is impossible to dry equally each piece of gut with filter paper thereby causing considerable inaccuracies in the weight of the loops. Consequently no attempt has been made to weigh the loops after each experiment.

d. Body weight:

The reliability of body weight and body surface area as methods of expressing rates of absorption depends on the accuracy of the relationship between these measurements and the mucosal surface area of the gut. It is not advisable to use body weight and body surface area if loops of gut, and not the whole gut, are employed to determine the rate of absorption of a substance.

Before it is possible to compare the relative rates of absorption in upper and lower loops of the small intestine it is necessary to express these rates in terms

of either unit gut length, unit mucosal surface area, or unit gut weight. The last has been abandoned in favour of expressing rates of absorption in terms of unit length and unit surface area.

Concentration:

For no apparent reason the concentration used by different workers in this field seems to vary widely, e.g., Cori (1926-27) employs solutions of amino acids up to 15%. Verzár, however, always used isosmotic solutions in his investigation into the absorption rates of hexoses and pentoses. A half isosmotic solution of the amino acids has been used in the present study since amino acids are much more sparingly soluble in water than sugars. With isosmotic and half isosmotic solutions it has been shown histologically that there is no danger of gross injury to the epithelial cells of the small intestine (Figs. 5 and 13).

Relative rates of absorption:

The results in Tables 4, 5, 6, 7 and 8 make it clear that phenylalanine has the greatest rate of absorption in the small intestine no matter whether the rate of absorption is expressed in mg. phenylalanine absorbed per cm. gut or mg. phenylalanine absorbed per sq. cm. mucosal surface area. Isoleucine has a slightly lower rate of absorption while glycine and alanine are absorbed

still more slowly - alanine being the slowest of all four amino acids.

Isoleucine:

The relative rates of absorption of isoleucine in the upper and lower loops of the small intestine of the rat are approximately equal and are not statistically different. The average absorption rate of isoleucine is 0.71 mg. per cm. gut in the upper loop and 0.70 mg. per cm. gut in the lower loop during an absorption period of 60 minutes. If, however, the absorption rates are expressed in mg. isoleucine absorbed per unit surface area there is a definite difference in the rates of absorption in upper and lower loops of the small intestine. The lower loop absorbs isoleucine at a greater rate than the upper loop, the lower absorbing 0.14 mg. per sq. cm. mucosal surface area while the upper loop absorbs 0.08 mg. per sq. cm. mucosal surface area in 60 minutes.

Phenylalanine:

In the case of phenylalanine, the rate of absorption in the upper loop is slightly less than that in the lower loop, but is not statistically different. If the rates of absorption are expressed in mg. per cm. gut the rate of absorption in the upper loop is 0.93 mg. phenylalanine absorbed per cm. gut and 0.98 mg.

phenylalanine absorbed per cm. gut in the lower loop. The time of absorption in both cases is 60 minutes. If the absorption rate is expressed in mg. phenylalanine absorbed per unit surface area the greater rate of absorption in the lower loop becomes more marked, the upper loop absorbing 0.11 mg. phenylalanine per sq. cm. mucosal surface area in 60 minutes while the lower loop absorbs 0.19 mg. phenylalanine per sq. cm. mucosal surface area in the same time.

Alanine:

On the other hand the reverse occurs in the relative absorption rates of alanine in upper and lower loops of the small intestine of the rat when the rate of absorption is expressed in mg. per cm. gut. The absorption rate of alanine in the upper loop is 0.61 mg. per cm. gut, and in the lower loop it is 0.53 mg. per cm. gut, i.e., greater absorption takes place in the upper loop. However, if the absorption rates are expressed in mg. alanine absorbed per unit mucosal surface area the general trend of more rapid absorption in the lower loop is apparent. The upper loop absorbs 0.07 mg. alanine per sq. cm. mucosal surface area in 60 minutes while the lower loop absorbs 0.10 mg. alanine per sq. cm. mucosal surface area in the same time. These values are not statistically different.

Glycine:

Similar variations occur in the relative

absorption rates of glycine in upper and lower loops of the small intestine of the rat. When the rate of absorption is expressed in mg. per cm. gut greater absorption takes place in the upper loop than in the lower loop --- 0.63 and 0.56 mg. glycine per cm. gut respectively. When the absorption rate is expressed in mg. glycine absorbed per unit surface area per hour greater absorption appears to take place in the lower loop --- 0.11 mg. glycine per sq. cm. mucosal surface area in 60 minutes while the upper loop absorbs 0.07 mg. glycine per sq. cm. mucosal surface area in the same time. These values are not statistically different.

It is evident from Tables 3, 4, and 8 that there is very little difference in the absorption rates per hour of isosmotic and half isosmotic glycine from the upper loops of the small intestine of the rat. The similarity is not quite so marked in the lower loops. In the upper loop of the small intestine of the rat 0.06 mg. isosmotic glycine and 0.07 mg. half isosmotic glycine are absorbed per sq. cm. mucosal surface area per hour, while in the lower loop the absorption rates are 0.07 mg. half isosmotic glycine and 0.11 mg. isosmotic glycine per sq. cm. mucosal surface area per hour. The absorption rate of isosmotic glycine is greater in the lower loop than in the upper when expressed in mg. per sq. cm. gut whereas the

converse is true when expressed in mg. per cm. gut. This confirms the findings of Cori (1926-27) who, working with rats, postulated that the rate of absorption of glycine was within wide limits independent of the absolute amount and concentration.

Milli molar rates of absorption:

Another way in which absorption rates have been expressed recently is by what is known as the milli molar rate of absorption. The milli molar rate of absorption is found by dividing the actual rate of absorption by the molecular weight of the amino acid in question. Comparison between milli molar rates of absorption of different amino acids are therefore independent of their respective molecular weights. Considering the results for upper loops in Tables 4, 5, 6, 7 and 8, it is obvious that half isosmotic glycine (molecular weight 75) has the greatest milli molar rate of absorption, namely 0.20. Alanine (molecular weight 89) is next with a milli molar rate of absorption of 0.18. Phenylalanine (molecular weight 165) has an even lower milli molar absorption rate — 0.15. Curiously enough isoleucine (molecular weight 131) has the lowest milli molar rate of absorption of the four amino acids.

In the lower loops glycine again has the highest

milli molar absorption rate --- 0.19, and isoleucine the lowest --- 0.14. In contrast to the upper loops, however, phenylalanine has a very slightly higher milli molar rate of absorption than alanine --- 0.16 and 0.15 respectively.

Graphs:

An examination of the graphs in Fig. 15 shows a general tendency for the rate of absorption of the amino acids in mg./60 mins. in both upper and lower loops to rise as the molecular weight increases. Similarly in Fig. 14 the rate of absorption of the amino acids in mg./60 mins. in both upper and lower loops rises as the apparent molal volume increases. The correlation coefficients of the relationship are r = 0.906, p = 0.10 - 0.05, in the upper loops which indicates that the relationship is barely significant, and r = 0.912, p = 0.10 - 0.05, in the lower loops which also indicates that the relationship is barely significant.

The shapes of the graph in Fig. 14 show a general correspondence to the shapes of the graphs in Fig. 15. After the point of intersection of the graphs in figures 14 and 15 there is a sharp increase in the gradients.

A study of the graphs in Fig. 16 shows that as the milli molar rate of absorption decreases the molecular weight increases. Similarly graphs in Fig. 17 indicate

that as the milli molar rate of absorption decreases the apparent molal volume increases. Again, the shapes of the graphs in Fig. 16 show a general correspondence to the shapes of the graphs in Fig. 17. There is the same tendency for a sharp increase in the gradient of the graphs after the point of intersection.

This sharp increase in gradient caused by the absorption rate of phenylalanine agrees remarkably well with the data for the chick plotted by Kratzer (1944) in Fig. 10. There is also a good correspondence between the shape of Kratzer's graph and the graphs given in Fig.17. Kratzer found the absorption rate of L.leucine in the chick whereas isoleucine is used in the present investigation. As the apparent molal volumes of isomers of the amino acids are the same (Cohn and Edsall, 1943) the shape of Kratzer's graph and of the graphs here may still be compared.

Comparison with other workers:

It is difficult to compare the above results with the findings of other workers as the Cori technique, using the whole of the small intestine, is involved. However, Laszt (1938) using 60 cm. loops and the same modification of Cori's technique as is employed in this thesis, gives the following results (Table 12) for isosmotic solutions of amino acids:-

TABLE 12.

Amino Acid.	Initial vol. per loop.	Length of loop.	Time of absorption.	Abs. mg/100g/hr.	Abs. %
Glycine.	6 c.c.	60 cm.	1 hr.	50.1	75
DL·Alanine.	6 c.c.	60 cm.	1 hr.	35.6	40
L·Isoleucine.	6 c.c.	60 cm.	1 hr.	31.8	23

The percentage results for glycine are not inconsistent with those given in Table 8. Lasst, however, used isosmotic DL alanine instead of L(+)alanine which is the naturally occurring form and might reasonably be absorbed at a slightly faster rate than the optically inactive form. He also found the absorption rate of isosmotic L-isoleucine was 23% whereas the absorption rate of half isosmotic D·isoleucine in the present investigation is 48.5%.

A. Glycine and alanine:

A survey of the work of other investigators shows a considerable difference in the rates of absorption of the various amino acids. There is quite general agreement that glycine and alanine are absorbed more rapidly than other acids. [Folin and Dennis (1912 a,c)

Levene and Kober (1908-09), Levene and Meyer (1909-10), Seth and Luck (1925), Johnston and Lewis (1928), and later Wilson and Lewis (1929).

It should be remembered, however, that all of the evidence from the earlier workers is indirect, being based on blood and urine analyses, and this involves a second variable, the ease of disposal of the amino acids by the tissues. Wilson and Lewis (1929) confirmed that the methods used by these earlier workers gave a fairly reliable picture of relative rates of absorption. Cori (1926-27) found that glycine was absorbed at a slightly faster rate than DL alanine. He reported a series of experiments in which he found the rate of absorption of glycine to be very similar to that of DL alanine. On the other hand, Wilson and Lewis (1929) claimed that glycine was absorbed at a much slower rate than DL or L(+)alanine.

Cori's findings were: glycine 48 mg/100g.rat/hr.
DL alanine 45mg/100g.rat/hr.

Wilson & Lewis' findings were: glycine 50 mg/100g.rat/hr.
DL alanine 73mg/100g.rat/hr.

In a later paper, Wilson (1932) claimed that L(+) alanine was absorbed at a faster rate than glycine but Höber and Höber (1938 a,b) and Laszt (1938) disagreed

with this work. They confirmed Cori's findings that glycine is absorbed at a faster rate than L(+)alanine.

In the present work glycine is found to be absorbed at a slightly higher rate than L(+)alanine. A consideration of Table 8 shows a close similarity between the results for glycine and L(+)alanine, especially in the upper loops where the percentage absorption and the absorption per sq. cm. mucosal surface area are 68% and 0.07 mg., and 64% and 0.07 mg. respectively. In the lower loops the corresponding figures are 66% and 0.11 mg. for glycine and 54% and 0.10 mg. for alanine. This is in agreement with the findings of Cori.

B. Phenylalanine:

It will be seen from Table 8 that L(-) phenylalanine has the greatest rate of absorption in both upper and lower loops whether expressed as mg. per cm. gut or mg. per sq. cm. mucosal surface area. This is interesting in view of the fact that glycine and alanine are invariably quoted as having the highest rates of absorption from the small intestine of the rat. No results appear to have been published for the absorption rates of phenylalanine from the small intestine of rats. However, working with the chick, Kratzer (1944) found that the sodium salt of DL phenylalanine was absorbed at a much faster rate than either of the half sodium salts of

glycine or DL·alanine (i.e. salts prepared by the addition of half the exact amount of sodium hydroxide calculated to convert all the amino acid to the sodium salt). He found the absorption rate of the glycine salt was 38.0 mg/100g/hr. and that of the DL·alanine salt was 43.3 mg/100g/hr. The absorption rate of the sodium salt of DL·phenylalanine is given as 56.2 mg/100g/hr.

6. Isoleucine:

From Table 8 it will be seen that isoleucine is absorbed at a slightly faster rate in both upper and lower loops than alanine and glycine but at a slower rate than phenylalanine, whether the rate is expressed in terms of cm. gut or per sq. cm. mucosal surface area. No data for absorption rates of D(-)isoleucine appears in the literature. Lasst (1938), however, gives the rate of absorption of isosmotic L - isoleucine as being slightly less than that of isosmotic DL·alanine and much less than that of glycine.

These data indicate that the four amino acids studied are apparently all readily absorbed from the small intestine of the rat. They also indicate that absorption in lower regions of the small intestine is equal to, if not greater than, absorption in the upper loops.

IV. SUMMARY.

The relative rates of absorption of glycine, L(+) alanine, L(-)phenylalanine and D(-)isoleucine in upper and lower loops of the small intestine of the anaesthetised rat were determined. These absorption rates were expressed as the milli molar absorption rate as well as in terms of mg. amino acid absorbed per cm. gut, and mg. amino acid absorbed per sq. cm. mucosal surface area.

When the absorption rates were expressed in terms of unit gut length glycine, L(+)alanine, D(-)isoleucine, but not L(-)phenylalanine were all absorbed more rapidly in the upper loop than in the lower loop. However, when the rates of absorption were expressed in terms of unit mucosal surface area glycine, L(+)alanine, D(-)isoleucine and L(-)phenylalanine appeared to be more rapidly absorbed in the lower loop than in the upper loop.

Data for the milli molar rates of absorption per hour and absorption rates in mg. per hour of the amino acids from upper and lower loops of the small intestine of the anaesthetised rat were plotted against molecular weight and apparent molal volume respectively and compared. As the absorption rate in mg. per hour of the amino acids increased the apparent molal volume and the molecular weight increased. As the apparent molal

volume and the molecular weight decreased the milli molar absorption rates of the amino acids per hour increased.

A statistical analysis was made of all results.

Isosmotic and half isosmotic glycine were absorbed at the same rate from upper and lower loops of the small intestine. The similarity was most striking in the upper loops but less marked in the lower loops.

-----oOo-----

S E C T I O N 'B'.

---oOo---

Rates of Absorption of Amino acids from upper and lower loops of the small intestine of Decerebrate Cats and of Cats under Urethane Anaesthesia.

I. Introduction.

II. Past work.

III. Present work.

a) Method.

b) Results.

c) Discussion.

IV. Summary.

----oOo----

Rates of Absorption of Amino acids from upper and lower loops of the small intestine of Decerebrate Cats and of Cats under Urethane Anaesthesia.

I. INTRODUCTION.

Absorption of sugars from the gut shows marked difference as between rat and cat. It is desirable to know if any similar species difference is apparent with respect to amino acids. Data for the absorption rates of various optically active amino acids and their isomers from the small intestine of rats are readily available but there is no such information for cats. It seems worth while, therefore, to measure the rates of absorption of optically active amino acids and their isomers from the small intestine of the decerebrate cat and from the small intestine of the cat under urethane anaesthesia. As in the case of mandelic acid, any differences in the rates of absorption between these amino acids and their respective isomers might be masked by the anaesthetic (Barry and Smith, 1943).

II. PAST WORK.

No references could be found to any systematic examination of the rate of absorption of amino acids from

the small intestine of the cat. The evidence, such as it is, for absorption of amino acids fromthe small intestine of the cat is mainly indirect.

The rates of absorption of amino acids from the intestinal tract have been reported for the rat, dog, rabbit, chick, and for man, and the levels of blood amino nitrogen in these animals during amino acid absorption have been determined, but few studies of this nature have been published for the cat.

Evidence for absorption of amino acids from the small intestine of the cat is mainly indirect. Folin and Denis et al (1912(a)) showed that it was possible by means of their new analytical methods (1912 d,e,f,g,h) to trace urea, glycine and pancreatic amino acid mixtures not only into the blood, but also into the general tissues of the body. The increasesin the non protein nitrogen of the blood and muscles obtained by these workers in absorption experiments with cats were large enough to account for practically all the nitrogenous material absorbed from the intestine. Folin and Denis did not claim to account exactly or quantitatively for all that had been absorbed, but were content to account for practically all the absorbed nitrogen. Their results made untenable and superfluous the hypothesis of

of immediate synthesis in the walls of the intestine.

Later (1912 (c)), Folin and Denis proved that the absorption of such amino acids as glycine, alanine, asparagine, creatine, creatinine and tyrosine from the small intestine of the cat was accompanied by the formation of Urea. They showed that food protein reaches the tissues of the body in the form of amino acids and those amino acids which are not needed for the rebuilding of broken down body material are not rebuilt either into protein or protoplasm, but are broken down and their nitrogen converted into urea. Thus there was virtually nothing left of the older teaching of Pflüger (1893) and Voit (1867) on this particular subject - that the food protein after absorption became living protoplasm before being destroyed or was decomposed as circulating protein.

Table 13 summarises the results obtained by Folin and Denis. They expressed their rates of absorption as mg. of nitrogen absorbed. I have calculated their results in terms of percentage amino acid absorbed and actual amount of amino acid absorbed. The periods of absorption, the dietetic history of the cats, the anaesthetics used, differed widely and in some cases one kidney had been removed or the blood supply to both kidneys cut off by ligatures.

TABLE 13.

Amino Acid.	Absorption.		Period.	Diet.	Fasted.	Anaesthetic.	Remarks.
10% Glycine.	3.04g.	30.4%	45min.	Meat.	40hrs.	Ether only.	Arteries and veins of the kidneys ligatured.
10% Glycine.	1.28g.	25.6%	30min.	Meat.	Nil hrs.	Ether/morphine.	- - -
10% Glycine.	4.91g.	49.1%	230min.	Meat.	28hrs.	Ether/chloretone.	- - -
10% Glycine.	0.85g.	28.3%	120min.	Rice & Cream. (low nitrogen).	24hrs.	Ether/morphine.	- - -
2g Tyrosine in 125cc sodium carbonate solution.	Very slow.		-	-	24 hrs.	Ether/morphine.	- - -
6% Creatinine.	2.21g.	37.8%	80min.	-	3days.	Ether/morphine.	Minus rt.kidney. Ureter of left kidney ligatured.
6% Creatinine.	2.48g.	42.5%	123min.	Rice & Cream. (low nitrogen).	Nil hrs.	Ether/morphine.	- - -
10% Asparagine.	2.16g.	42.4%	20min.	Well fed.	24hrs.	Ether only.	- - -
10% Asparagine.	4.98g.	48.9%	120min.	-	24hrs.	-	Arteries and veins of the kidneys ligatured.

- Continued -

TABLE 13 (Contd).

Amino Acid.	Absorption.		Period.	Diet.	Fasted.	Anaesthetic.	Remarks.
5.75% Creatine.	1.21gs.	24.3%	44min.	Rice & Cream. (low nitrogen).	Nil hrs.	Ether/Morphine.	Minus rt. kidney.
4.8% Creatine.	1.56g.	30.0%	129min.	Eggs.	26 hrs.	Ether/Morphine.	Kidneys undisturbed.
13.62% Alanine.	3.81g.	40.0%	168min.	Meat.	24 hrs.	Ether/Morphine.	- - -
90.8% Alanine.	2.85%	91.6%	182min.	Rice & Cream (low nitrogen).	Nil hrs.	Ether/Morphine.	

The only other observations concerning the absorption of amino acids from the small intestine of the cat are those of Bolton and Wright (1937). These authors investigated the concentrations of amino acids in the whole blood and plasma in the carotid artery, superior mesenteric vein, hepatic vein, inferior vena cava and in the lymph from the cisternal chyli or thoracic duct during the conditions of fasting, starvation and feeding with peptone, egg white, lobster, cockles, haddock and milk. Their observations led them to postulate that the absorption of amino acids from the small intestine into the capillaries and lymphatics was in accordance with the physical law of diffusion: that there was no clear evidence of selective activity on the part of the capillary or lymphatic endothelium.

III. PRESENT WORK.

(a) Methods:

The method employed is similar to that described in Section A and is basically that of Cori (1925). A known quantity of a solution of amino acid of given strength is placed in the gut and the residual quantity determined after a time interval. All the cats were kept on a complete diet of fish, meat, and milk. Since they were invariably infected with tapeworms, the

animals were treated with arecoline hydrobromide when first received - dosage 0.1 mg. in milk, followed by castor oil. The cats were placed on a diet of milk and water for at least three days and were fasted 24 hours before use. They were then lightly etherised prior to decerebration or to anaesthetising with urethane. Decerebrate cats were used to avoid interference from anaesthesia.

Decerebrate cats:

Decerebration was carried out by a modification of the Landis (1930) and Schmidt (1923) methods. Cats weighing from 1.7 to 4 kilos were lightly etherised, the trachea cannulated and the carotid arteries ligatured. The skin of the head was incised and the skull bared. Trephine openings (two) were made in the cranium and the cerebral hemispheres carefully scooped out with a spoon and with cotton wool swabs. The loss of blood was very slight. Artificial respiration was seldom required at this stage and spontaneous movements were absent. Of the 62 animals prepared in this way, only three died. The cause of death appeared to be heart failure brought on by excess of ether; in two of the animals which died an advanced stage of pregnancy was observed.

Anaesthetised cats:

When the cats were anaesthetised with urethane a slightly different procedure from that used in rats

(Section A) was adopted. The weight of the cats varied from 1 to 4 kilos. A cannula was inserted into the trachea and the femoral vein of the left leg was exposed. A solution of 5% urethane in mammalian Ringer was slowly run into the femoral vein using a number "0" ball-pointed metal cannula. The injection of the urethane solution usually occupied about an hour. It was found that the metal cannula was more easily inserted into the vein after it had been buffed down to the shape of a pear. The dosage of urethane was 1.25 g. per Kilo.cat. In some cases it was necessary to increase the dose slightly to obtain the required depth of anaesthesia. Sufficient depth of anaesthesia was usually reached an hour after commencing the injection of the urethane. During this period the cat was kept on a warm operating board to ensure that its temperature remained constant at 38°C. If the urethane solution was administered too rapidly death very often ensued. The ideal rate was found to be not more than 1.5 c.c. of the urethane solution per minute.

Operative procedure:

The abdomen was opened in the mid line and ligatures of narrow tape, which did not cut into the gut wall, tied as described in Section A for the rat (see Fig.11). Two loops of the small intestine, of approximately the same length (30 cm. each) were measured off.

Such loops were each roughly a quarter of the entire length of the small intestine. The small intestine was washed out with warm saline solution (1% w/v) at 38°C and the loose ligatures tied as described in Section A. From a burette a known amount of an isosmotic or half isosmotic amino acid solution at 38°C was run into each loop of the small intestine. The loops were tied off, the abdomen closed and the animal left in warm surroundings for 40 minutes, the period of absorption.

The animal was killed by bleeding and the entire small intestine excised, care being taken to avoid stretching the loops. Each loop was measured by placing it against a vertical centimetre scale. The contents of each of the loops were washed into a large filter funnel draining into a 600 c.c. beaker. Each loop was slit along its whole length and the mucosa thoroughly washed with warm saline solution (1% w/v). The total volume of the contents of the loops and the solution used to wash them amounted to about 200 c.c. in each case.

Estimation of recovered material

The method of estimation of the recovered material was similar to that described in the rat, Section A.

Preparation of solutions of amino acids:

The amino acids used were glycine, L(+) alanine, $[\alpha]_D = +14.70^\circ$ (0.97N HCl), L(-) phenylalanine, $[\alpha]_D = -35.14^\circ$ (water), and D(-) isoleucine, $[\alpha]_D = -10.50^\circ$ (water) supplied by Roche. Solutions, half isosmotic and isosmotic with the blood, were made up 24 hours before use.

Bacteria of the gut:

As in the rat, Section A, estimations were made to determine if the bacteria of the gut utilised the amino acids presented to them. Incubation at 37°C for 1 hour of a known volume of amino acid solution with 1% (w/v) saline which had been perfused through the lumen of the small intestine showed that the bacteria of the gut do not use the amino acid to any detectable extent.

Glycine as the sodium salt:

To a known solution of glycine was added the exact amount of sodium hydroxide calculated to convert all the glycine to the sodium salt. Since the sodium salts of the amino acids are more soluble than the free acids in water it could be foreseen that this procedure might have to be resorted to later in the case of amino acids sparingly soluble in water. The pH of the solution of the sodium salt of glycine was in the region of 10.0 whereas that of an isosmotic solution of glycine was 7.4.

Figs. 18 and 19.

Fig. 18 shows the appearance of the lower loop of the small intestine of the cat in transverse section after a solution of isosmotic glycine (sodium salt) had been placed in the gut lumen for 40 minutes. The lumen is packed with cellular debris and there is some injury to the gut epithelium, especially at the tips of the villi. X 5.

Fig. 19 is a photomicrograph of the lower loops of the small intestine of the anaesthetised cat after absorption of isosmotic glycine (sodium salt) had proceeded for 40 minutes. Desquamation of the epithelial cells of the villi is severe. X 96.

Figure 18.

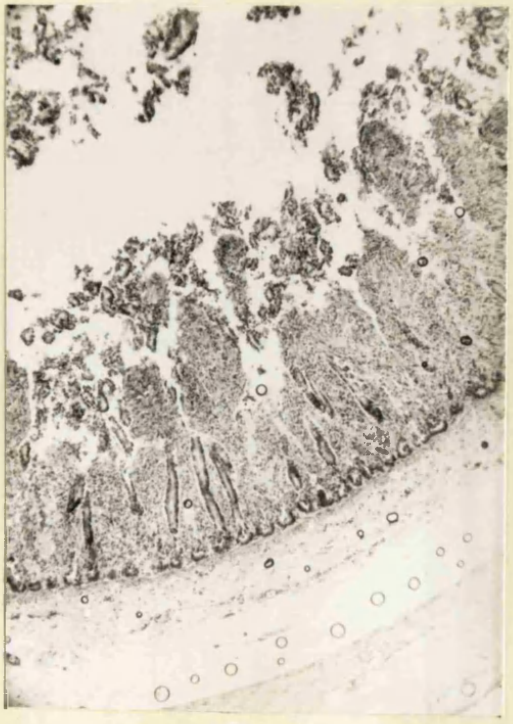


Figure 19.

Histology:

It was thought necessary to examine the epithelium of the small intestine histologically in case the isosmotic solution of the sodium salt of glycine should cause epithelial desquamation. At the end of each experiment a small piece of the intestinal loop was fixed in Bouin's fluid, embedded in paraffin, cut at 8μ and stained with Haematoxylin and Orange G. Microscopical examination showed desquamation of the epithelial cells of the villi (Figs. 18 and 19). Rather than use sodium salts of amino acids sparingly soluble in water it was considered preferable to employ half isosmotic solutions if the latter could be shown to cause no damage to the epithelial cells. Microscopical examination revealed, however, no gross injury to the epithelium during absorption of half isosmotic solutions.

(b) Results:

Presentation of Tables:

As in the case of the rat (see Table 2) the results of each experiment were recorded in a protocol. In Tables 14 to 27 all the data necessary for the determination of the relative absorption values in upper and lower loops of the small intestine of the cat are recorded. Tables 14 and 15 record the results obtained with isosmotic glycine in decerebrate cats and cats under urethane

anaesthesia respectively. Nine cats were employed in the first case and five in the latter. In all of these the time of absorption was 40 minutes. Table 14 also shows the rates of absorption of isosmotic glycine in four decerebrate cats over an absorption period of 1 hour.

Tables 16 and 17 give results for the absorption in 40 minutes of isosmotic glycine sodium salt in upper and lower loops of the small intestine of the cat. Table 16 shows the absorption values from loops of the small intestine of five cats under urethane anaesthesia and Table 17 the result for the same period from the small intestine of one decerebrate animal.

In Table 18 are given the absorption values from loops of the small intestine of four decerebrate cats of an isosmotic solution of the sodium salt of glycine to which N/1 hydrochloric acid was added to give a solution having a pH 7.4. In all of these the time of absorption was 40 minutes.

Table 19 records the absorption results obtained with a mixture of equal parts of half isosmotic glycine and half isosmotic glucose from the small intestine of five cats under urethane anaesthesia. The time of absorption was 40 minutes.

Similarly Tables 20 and 21, 22 and 23, 24 and 25, 26 and 27 show the absorption values for half isosmotic solutions of glycine, L - phenylalanine, L alanine, and D - isoleucine respectively. Tables 20, 22, 24 and 26 refer to cats under urethane anaesthesia; Tables 21, 23, 25 and 27 refer to decerebrate cats. The time of absorption in all of these was 40 minutes.

Results from all experiments not wholly free from technical fault are omitted.

Period of absorption:

Results given for isosmotic glycine after an interval of 1 hour (Table 14) indicate that practically all the amino acid disappears from the gut lumen. An absorption period of 40 minutes was therefore chosen. Such an interval allowed fully half the amino acid to disappear from the gut lumen. This period of absorption was found suitable for determining absorption rates of half isosmotic solutions of amino acids.

Column headings:

The column headings are the same as those described in the case of the rat in Section A.

The absorption rate per sq. cm. gut is obtained by dividing the actual absorption by Wood's (1944) figures for the mucosal surface area of the jejunum and ileum of the cat. These mean figures are, in the case of the cat:-

Jejunum 49.5 sq.cm. / cm. serosal length.
Ileum 39.5 sq.cm. / cm. serosal length.

In Table 28 the average absorption values, the actual absorption, the rate of absorption per unit length, the rate of absorption per unit surface area and the milli molar absorption rate of isosmotic and half isosmotic amino acids over a period of 40 minutes are recorded for upper and lower loops of the small intestine of the cat.

Graphs:

In figure 20 rates of absorption in mg. per 40 minutes of half isosmotic solutions of glycine, L(+) alanine, L (-) phenylalanine and D (-) isoleucine from upper and lower loops of the small intestine of decerebrate cats and of cats anaesthetised with urethane are plotted against molecular weight.

Similarly in Fig. 21, the rates of absorption in mg. per 40 minutes of half isosmotic solutions of glycine, L (+) alanine, L (-) phenylalanine and D (-) isoleucine from upper and lower loops of the small intestine of decerebrate and of anaesthetised cats are plotted against

the apparent molal volumes of these amino acids.

In Fig. 22 the milli molar rates of absorption of half isosmotic solutions of glycine, L(+)alanine, L(-) phenylalanine and D(-) isoleucine from upper and lower loops of the small intestine of decerebrate and of anaesthetised cats are plotted against molecular weight.

In Fig. 23 the milli molar rates of absorption of half isosmotic solutions of glycine, L(+) alanine, L(-) phenylalanine and D(-) isoleucine from upper and lower loops of the small intestine of decerebrate cats and cats anaesthetised with urethane are plotted against the apparent molal volumes of these amino acids.

Statistical Analysis of Results:

The actual absorption values of glycine, L(+) alanine, L(-) phenylalanine and D(-)isoleucine in upper and lower loops of the small intestine of the cat, given in Tables 14 to 27, are compared by the statistical methods described by Chambers (1946). In each Table the absorption values in the upper loop are compared with those in the lower loop of the anaesthetised animal and of the decerebrate animal. The absorption values in upper and lower loops of the anaesthetised animal are also compared with those of the decerebrate animal. In all cases the significance of the difference between the

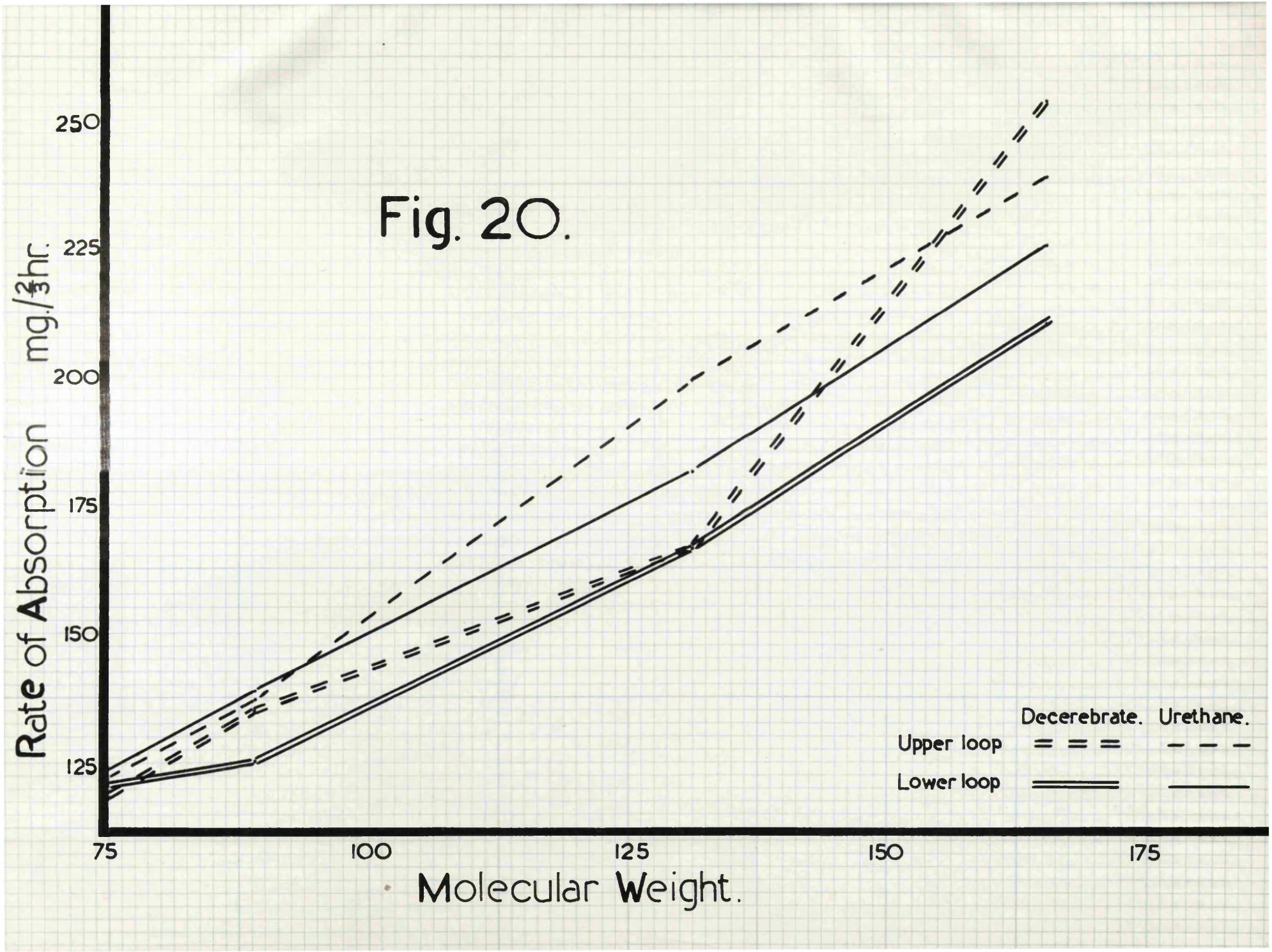


Fig. 20.
Rate of Absorption mg./⅓hr.
250
225
200
175
150
125
75
100
125
150
175
Molecular Weight.
Decerebrate.
Urethane.
Upper loop
Lower loop

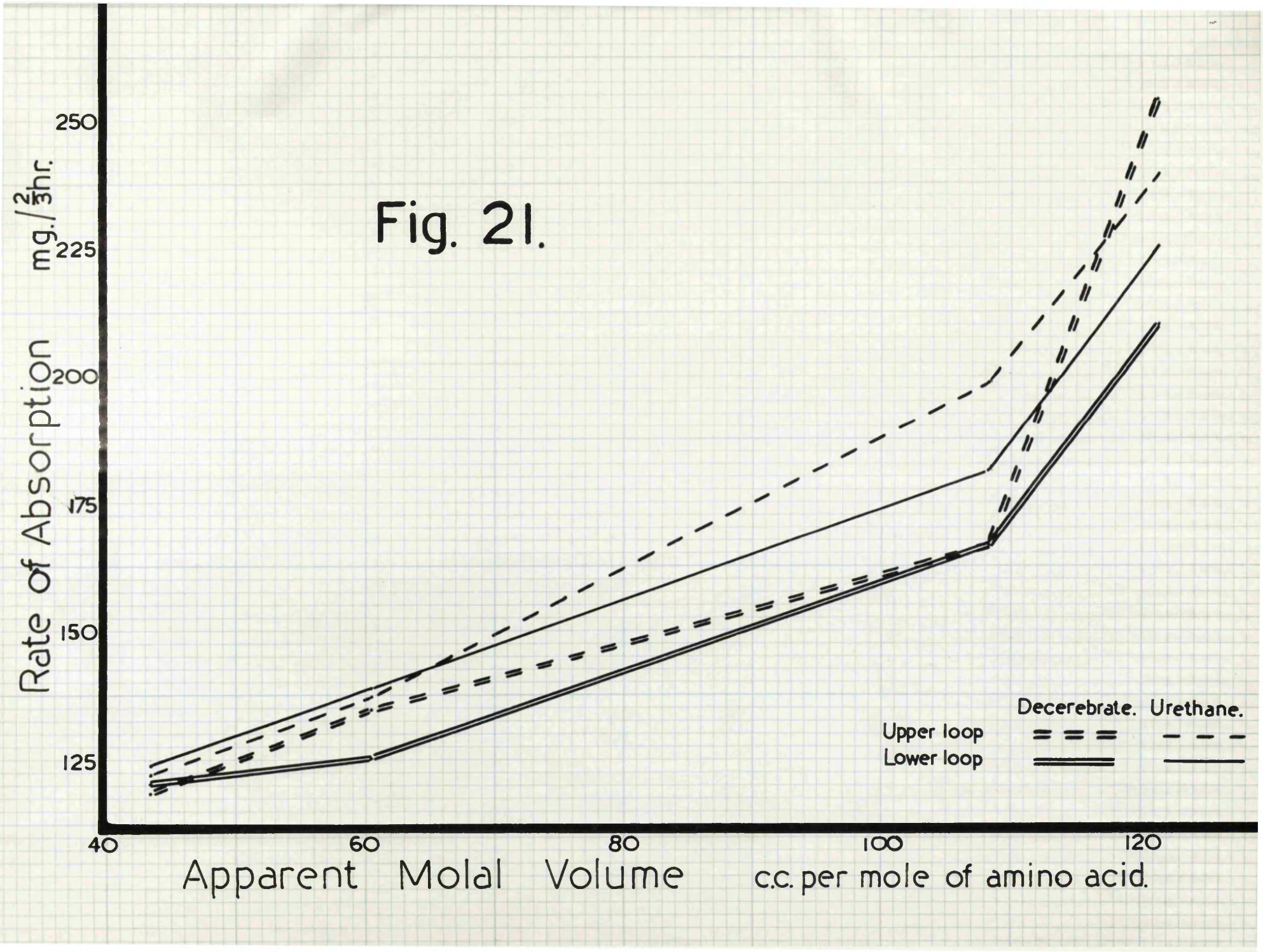


Fig. 21.
Rate of Absorption mg./2/3hr.
250
225
200
175
150
125
40
60
80
100
120
Apparent Molal Volume c.c. per mole of amino acid.
Decerebrate. Urethane.
Upper loop
Lower loop

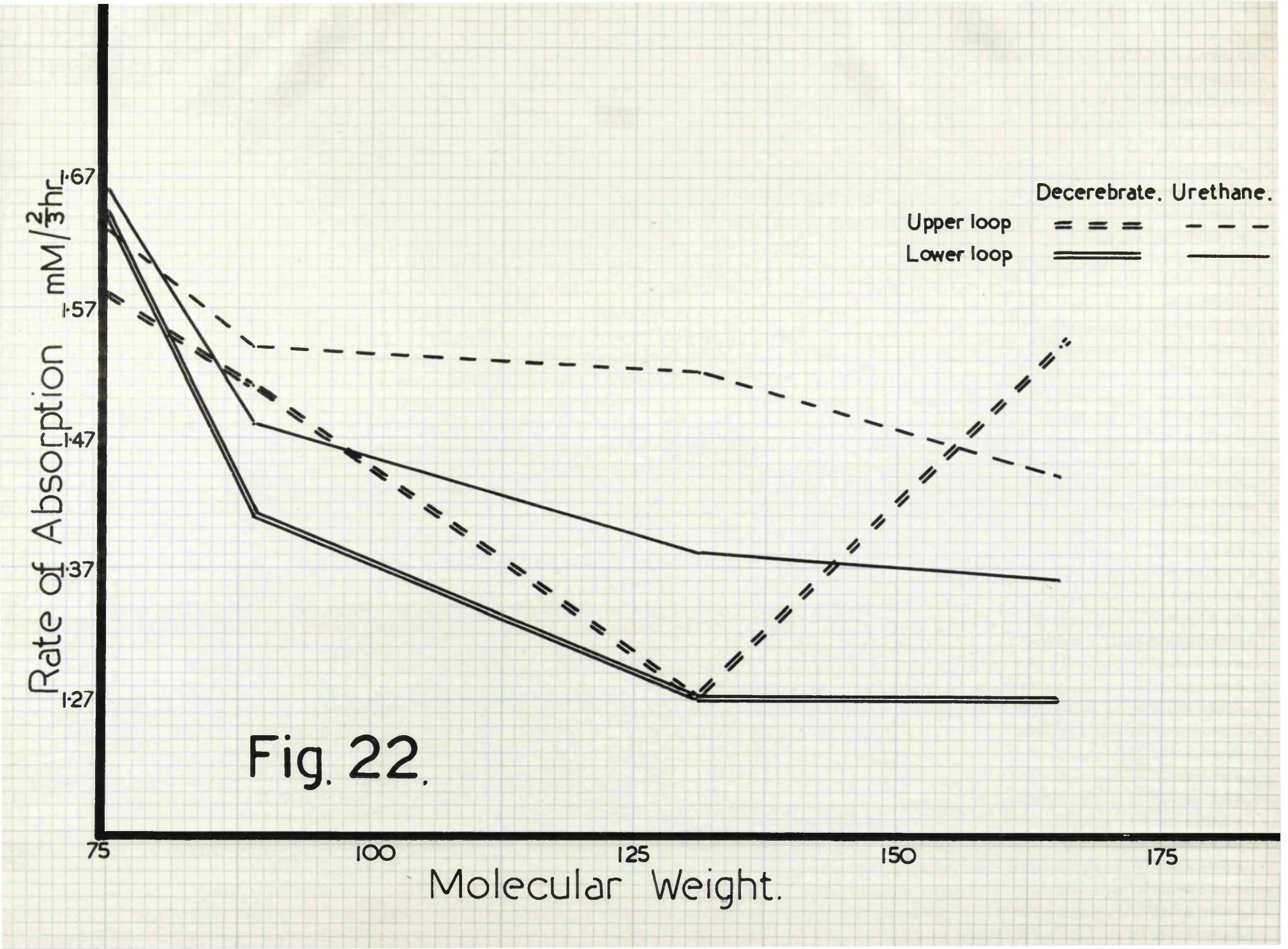

Decerebrate. Urethane.
Upper loop
Lower loop
Rate of Absorption mM/2/3hr.
1·67
1·57
1·47
1·37
1·27
75
100
125
150
175
Molecular Weight.

Fig. 22.

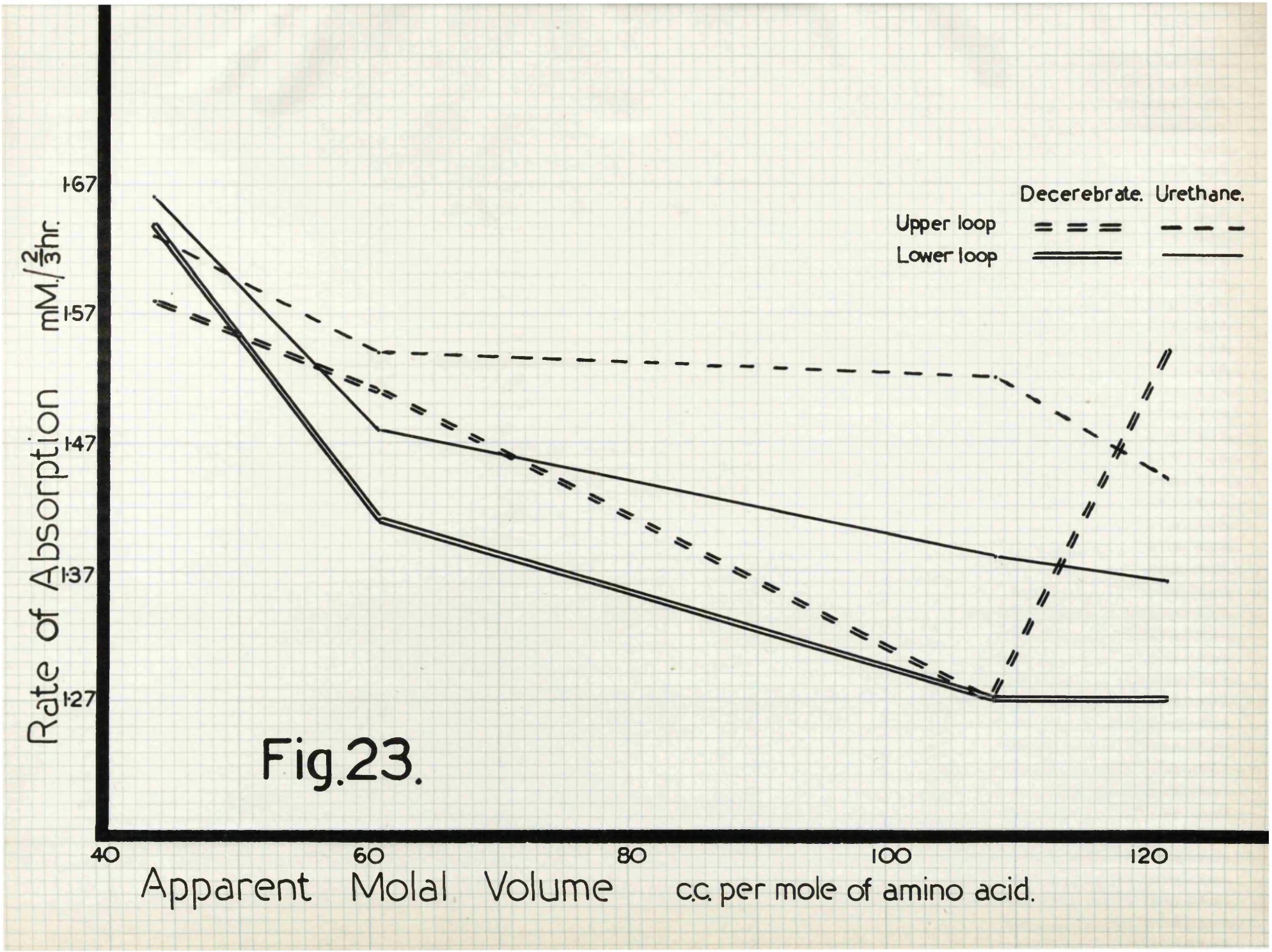

Decerebrate. Urethane.
Upper loop
Lower loop
Rate of Absorption mM./2/3hr.
1·67
1·57
1·47
1·37
1·27
40
60
80
100
120
Apparent Molal Volume c.c. per mole of amino acid.

Fig.23.

TABLE 14

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
2/6/47	Cat	F	2250	60	10.0	20.0	192	88	9.58	.19	2.55	10.0	26.0	217	99	8.35	.23	2.89
3/6/47	Cat	F	2150	60	10.0	24.0	212	97	8.85	.18	2.83	10.0	23.0	214	98	9.31	.26	2.85
4/6/47	Cat	F	2200	60	10.0	25.0	202	93	8.07	.16	2.69	10.0	32.0	209	96	6.52	.18	2.78
5/6/47	Cat	F	2320	60	15.0	30.0	327	100	10.89	.22	4.36	11.0	24.0	230	96	9.58	.27	3.07
11/6/47	Cat	M	3085	40	15.0	30.0	260	80	8.67	.18	3.47	15.0	22.0	217	67	9.88	.28	2.90
12/6/47	Cat	F	3100	40	15.0	27.0	277	85	10.28	.21	3.70	15.0	18.0	158	48	8.78	.25	2.11
13/6/47	Cat	F	2330	40	15.0	22.0	277	85	12.58	.25	3.69	11.3	21.0	182	74	8.65	.24	2.42
18/6/47	Cat	F	3450	40	14.0	16.0	204	67	12.74	.26	2.72	10.0	33.0	159	73	4.81	.14	2.12
21/6/47	Cat	F	4125	40	15.0	28.0	270	84	9.64	.19	3.60	15.0	21.0	258	79	12.27	.35	3.44
15/8/47	Cat	M	2725	40	15.0	25.0	262	80	10.48	.22	3.47	16.0	28.0	247	71	8.82	.25	3.30
16/8/47	Cat	F	1980	40	15.0	26.0	220	67	8.46	.17	2.91	16.0	24.0	290	83	12.09	.34	3.87
17/8/47	Cat	F	2175	40	15.0	27.0	240	73	8.88	.18	3.20	15.0	26.0	265	81	10.17	.29	3.53
18/8/47	Cat	F	2575	40	15.0	25.0	273	85	10.92	.22	3.61	13.0	28.0	229	81	8.17	.23	3.05

GLYCINE.

TABLE 15

Date	Animal	Sex	Weight	Time of absorption	UPPER LOOP							LOWER LOOP						
					Initial vol. per loop	Length of gut.	Absorption					Initial vol. per loop	Length of gut.	Absorption				
							Actual		Per cm. gut	Per sq. cm. gut.	Actual Mol. Wt.			Actual		Per cm. gut.	Per sq. cm. gut.	Actual Mol. Wt.
			gm.	min.	cc.	cm.	mg	%.	mg.	mg.		cc.	cm.	mg.	%.	mg.	mg.	
20/6/47	Cat	F	2550	40	15.0	24.0	311	95	12.95	.26	4.14	15.0	26.0	255	78	9.81	.28	3.40
26/6/47	Cat	F	2740	40	17.0	28.0	301	81	10.74	.22	4.01	17.0	29.0	218	59	7.52	.21	2.91
3/7/47	Cat	M	3950	40	16.0	25.0	316	91	12.63	.26	4.21	15.0	22.0	209	64	9.49	.27	2.78
7/7/47	Cat	F	2280	40	15.0	25.0	294	90	11.75	.24	3.92	14.0	22.0	194	63	8.80	.25	2.58
14/7/47	Cat	F	2350	40	16.0	28.0	320	92	11.44	.23	4.27	15.0	30.0	249	76	8.29	.23	3.32

GLYCINE.

TABLE 16

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual / Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual / Mol. Wt.
18/7/47	Cat	M	3260	40	17.0	32.0	139	30	4.35	.09	1.44	16.0	30.0	116	26	3.86	.11	1.19
21/7/47	Cat	M	2850	40	13.1	28.0	76	21	2.70	.05	0.78	13.0	31.0	105	29	3.38	.10	1.08
22/7/47	Cat	M	3125	40	16.0	28.5	164	37	5.76	.12	1.69	16.0	34.0	166	38	4.88	.14	1.71
30/7/47	Cat	F	1800	40	17.0	30.0	63	13	2.09	.04	0.65	17.0	27.0	111	24	4.11	.12	1.14
31/7/47	Cat	F	1925	40	10.5	20.0	26	9	1.31	.03	0.27	12.5	27.0	102	29	3.76	.11	1.05

SODIUM SALT OF GLYCINE.

TABLE 17

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
21/8/47.	Cat	F.	4125	40	15.0	22.0	71	17	3.23	.07	0.73	15.0	21.0	258	79	12.27	.35	3.44

SODIUM SALT OF GLYCINE.

(Glycine in lower loop).

TABLE 18

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
15/9/47	Cat	M	2725	40	16.0	27.0	394	89	14.61	.30		13.0	28.0	228	80	8.16	.23	3.05
16/9/47	Cat	F	1980	40	16.0	23.0	399	90	17.35	.35		16.0	24.0	290	83	12.09	.34	3.87
17/9/47	Cat	F	2175	40	11.0	27.0	265	87	9.80	.20		16.0	28.0	249	71	8.83	.25	3.31
18/9/47	Cat	F	2575	40	13.0	27.0	312	87	11.56	.23		15.0	26.0	264	81	10.14	.28	3.52

SODIUM SALT OF GLYCINE

(Glycine in lower loop)

TABLE 19

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
18/12/47	Cat	M	3500	40	22.0	28.0	113	47	4.04	.08	1.51	15.5	30.0	112	66	3.74	.11	1.50
30/12/47	Cat	M	2840	40	15.0	36.0	96	59	2.66	.05	1.28	15.0	28.0	106	65	3.80	.11	1.42
7/10/48	Cat	M	2825	40	15.0	23.5	126	77	5.35	.11	1.68	16.0	28.5	129	79	4.71	.13	1.71
29/9/48	Cat	M	2840	40	15.0	29.5	139	85	4.71	.10	1.85	13.0	23.5	120	85	5.09	.14	1.60
28/9/48	Cat	F	2360	40	15.0	27.0	109	67	4.03	.08	1.45	15.0	30.0	105	64	3.50	.10	1.40

GLYCINE + GLUCOSE

TABLE 20

Date	Animal + Sex	Weight gm.	Time of absorption min.	UPPER LOOP							LOWER LOOP						
				Initial vol. per loop cc.	Length of gut. cm.	Absorption					Initial vol. per loop cc.	Length of gut. cm.	Absorption				
						Actual mg	%	Per cm. gut mg.	Per sq. cm. gut. mg.	Actual Mol. Wt.			Actual mg.	%	Per cm. gut. mg.	Per sq. cm. gut. mg.	Actual Mol. Wt.
14/1/48	Cat M	1520	40	16.0	27.0	141	81	5.22	.11	1.88	16.0	26.0	121	69	4.64	.13	1.61
16/1/48	Cat M	3250	40	12.5	25.0	85	63	3.41	.07	1.14	16.0	31.0	119	68	3.84	.11	1.69
26/1/48	Cat M	2375	40	15.0	-	152	93	-	-	2.02	15.0	-	140	86	-	-	1.87
27/1/48	Cat M	3420	40	15.0	28.0	122	74	4.34	.09	1.62	15.0	32.0	132	81	3.85	.11	1.76
10/2/48	Cat F	2075	40	12.5	26.0	112	82	4.30	.09	1.49	15.0	30.0	120	90	4.01	.11	1.60
1/3/48	Cat F	3740	40	15.0	30.0	131	80	4.36	.09	1.74	15.0	31.0	123	81	3.95	.11	1.63
4/3/48	Cat F	1000	40	14.0	35.0	121	80	3.47	.07	1.62	13.0	34.0	133	87	3.91	.11	1.77
8/3/48	Cat F	1190	40	14.0	-	114	75	-	-	1.52	14.0	-	110	72	-	-	1.47

GLYCINE

TABLE 21

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual / Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual / Mol. Wt.
11/2/48	Cat	F	1850	40	16.0	-	137	79	-	-	1.85	14.0	-	124	81	-	-	1.65
17/2/48	Cat	F	2490	40	11.8	-	105	81	-	-	1.38	13.0	-	120	85	-	-	1.60
18/2/48	Cat	F	2325	40	13.0	31.0	117	82	3.76	.08	1.55	13.0	29.0	112	79	3.86	.11	1.49
19/2/48	Cat	M	1730	40	14.0	-	114	75	-	-	1.52	15.0	-	114	70	-	-	1.52
24/2/48	Cat	M	3125	40	15.0	-	131	80	-	-	1.74	16.0	-	137	79	-	-	1.83
26/2/48	Cat	M	1850	40	14.0	28.0	110	71	3.93	.08	1.47	14.0	30.0	117	77	3.90	.11	1.56

GLYCINE.

TABLE 22

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual / Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual / Mol. Wt.
26/11/47	Cat	M	2725	40	14.0	28.0	248	75	8.86	.18	1.50	15.0	24.0	239	68	9.94	.28	1.44
8/12/47	Cat	M	3875	40	12.0	24.0	226	80	9.43	.19	1.37	13.0	26.0	227	74	8.73	.25	1.38
16/12/47	Cat	M	2505	40	15.0	29.0	313	83	10.78	.22	1.89	11.9	29.0	175	63	6.03	.17	1.06
12/3/48	Cat	M	3290	40	14.0	30.0	239	73	7.96	.16	1.45	14.0	32.0	174	53	5.44	.15	1.05
18/3/48	Cat	F	3100	40	14.3	-	194	58	-	-	1.17	15.0	-	258	73	-	-	1.56
19/3/48	Cat	F	3000	40	14.0	-	273	83	-	-	1.65	13.0	-	306	80	-	-	1.85
22/3/48	Cat	M	1780	40	13.0	30.0	210	69	6.99	.14	1.27	13.7	-	225	64	-	-	1.36
23/3/48	Cat	M	1720	40	14.0	29.0	203	57	9.27	.18	1.23	14.0	28.0	199	56	7.09	.20	1.20

PHENYLALANINE.

TABLE 23

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual / Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual / Mol. Wt.
29/3/48	Cat	M	2150	40	13.0	28.0	220	73	7.95	.16	1.35	15.0	34.0	177	50	5.21	.15	1.07
30/3/48	Cat	M	1950	40	11.0	29.0	214	83	7.39	.15	1.30	12.0	30.0	230	81	7.66	.22	1.39
31/3/48	Cat	M	4000	40	15.0	23.0	276	78	12.00	.24	1.67	15.0	30.0	195	55	6.51	.18	1.18
1/4/48	Cat	M	2700	40	12.3	31.0	218	76	7.03	.14	1.32	12.0	28.0	154	54	5.49	.16	0.93
5/4/48	Cat	F	2500	40	14.0	30.0	261	79	8.70	.18	1.58	14.0	-	239	73	-	-	1.48
6/4/48	Cat	M	2000	40	15.0	-	287	81	-		1.74	15.0	-	249	71	-	-	1.51
8/4/48	Cat	M	2600	40	15.0	29.0	298	84	10.26	.21	1.80	15.0	32.0	229	64	7.14	.20	1.39

PHENYLALANINE

TABLE 24

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual / Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual / Mol. Wt.
12/5/48	Cat	M	2150	40	13.0	28.0	88	53	3.14	.06	0.99	12.5	29.0	108	69	3.74	.11	1.22
27/5/48	Cat	F	2250	40	13.9	29.0	132	75	4.56	.09	1.48	14.0	25.0	130	73	5.20	.15	1.46
1/6/48	Cat	M	2925	40	16.0	35.0	156	77	4.44	.09	1.75	16.0	29.0	148	73	5.09	.14	1.66
2/6/48	Cat	M	2680	40	15.0	29.0	139	73	4.78	.10	1.56	14.0	25.0	127	71	5.07	.14	1.42
3/6/48	Cat	M	2770	40	16.0	33.0	166	82	5.04	.10	1.87	16.0	38.0	168	83	4.41	.12	1.88
4/6/48	Cat	M	3230	40	14.0	21.0	143	80	6.80	.14	1.60	15.0	31.0	147	77	4.75	.13	1.65

ALANINE.

TABLE 25

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut. cm.	UPPER LOOP: Absorption: Actual mg	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
12/4/48	Cat	M	3475	40	15.0	33.0	162	85	4.90	.10	1.81	15.0	27.0	178	93	6.59	.19	2.00
14/4/48	Cat	M	3625	40	15.0	27.0	137	72	5.07	.10	1.54	15.0	27.0	151	79	5.59	.16	1.70
16/4/48	Cat	M	3750	40	15.0	34.0	85	44	2.49	.05	0.95	15.0	28.0	125	65	4.45	.13	1.40
19/4/48	Cat	F	2050	40	16.0	29.0	118	58	4.07	.08	1.32	16.0	32.0	124	61	3.86	.11	1.39
21/4/48	Cat	F	2450	40	16.0	29.0	144	71	4.97	.10	1.62	15.0	30.0	102	54	3.40	.10	1.15
22/4/48	Cat	M	3630	40	16.0	25.0	135	67	5.42	.11	1.52	15.0	25.0	107	56	4.29	.12	1.20
6/5/48	Cat	F	3090	40	17.6	27.0	163	74	6.02	.13	1.83	15.0	25.0	92	48	3.66	.10	1.03

ALANINE

TABLE 26

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP Initial vol. per loop cc.	UPPER LOOP Length of gut cm.	UPPER LOOP Absorption Actual mg	UPPER LOOP Absorption %	UPPER LOOP Absorption Per cm. gut mg.	UPPER LOOP Absorption Per sq. cm. gut. mg.	UPPER LOOP Absorption Actual / Mol. Wt.	LOWER LOOP Initial vol. per loop cc.	LOWER LOOP Length of gut cm.	LOWER LOOP Absorption Actual mg.	LOWER LOOP Absorption %	LOWER LOOP Absorption Per cm. gut. mg.	LOWER LOOP Absorption Per sq. cm. gut. mg.	LOWER LOOP Absorption Actual / Mol. Wt.
7/6/48	Cat	F	2125	40	15.0	30.0	206	73	6.86	.14	1.57	15.0	32.0	177	63	5.55	.16	1.35
11/6/48	Cat	M	1425	40	16.0	28.0	215	70	7.68	.16	1.64	13.0	28.0	168	64	5.99	.17	1.28
18/6/48	Cat	M	3530	40	15.0	35.0	191	68	5.44	.11	1.45	13.0	27.5	186	77	6.76	.19	1.42
22/6/48	Cat	M	2500	40	15.0	28.5	216	77	7.58	.15	1.65	16.0	28.5	218	73	7.64	.22	1.66
23/6/48	Cat	M	3750	40	15.0	27.5	155	55	5.63	.11	1.18	15.0	27.5	137	49	4.98	.14	1.05
24/6/48	Cat	M	2600	40	15.0	28.0	213	76	7.60	.15	1.62	14.5	28.0	201	74	7.16	.20	1.53

ISOLEUCINE

TABLE 27

Date	Animal	Sex	Weight gm.	Time of absorption min.	UPPER LOOP: Initial vol. per loop cc.	UPPER LOOP: Length of gut cm.	UPPER LOOP: Absorption: Actual mg.	UPPER LOOP: Absorption: %	UPPER LOOP: Absorption: Per cm. gut mg.	UPPER LOOP: Absorption: Per sq. cm. gut. mg.	UPPER LOOP: Absorption: Actual Mol. Wt.	LOWER LOOP: Initial vol. per loop cc.	LOWER LOOP: Length of gut. cm.	LOWER LOOP: Absorption: Actual mg.	LOWER LOOP: Absorption: %	LOWER LOOP: Absorption: Per cm. gut. mg.	LOWER LOOP: Absorption: Per sq. cm. gut. mg.	LOWER LOOP: Absorption: Actual Mol. Wt.
5/7/48	Cat	M	3000	40	15.3	28.0	151	53	5.40	.11	1.15	15.0	32.0	187	67	5.85	.17	1.43
8/7/48	Cat	M	2050	40	15.0	29.0	182	65	6.24	.13	1.39	15.0	35.0	174	62	4.98	.14	1.33
13/7/48	Cat	M	3000	40	15.0	27.0	195	69	7.21	.15	1.49	15.0	30.0	160	57	5.34	.15	1.22
15/7/48	Cat	F	2825	40	14.3	28.0	134	49	4.77	.10	1.02	15.0	27.0	149	53	5.51	.16	1.13
16/7/48	Cat	M	3125	40	15.0	31.0	196	70	6.33	.13	1.50	15.0	30.0	127	56	4.22	.12	0.97
19/7/48	Cat	M	3850	40	15.0	30.0	140	50	4.67	.09	1.07	15.0	23.0	201	72	8.72	.25	1.53

ISOLEUCINE

TABLE 28.

ABSORPTION.
Mean Values.

Amino acid used.	Time of Absorption.	UPPER LOOP. Actual.	%	per cm. gut.	per sq.cm. gut.	Actual/M.	LOWER LOOP. Actual.	%	per cm. gut.	per sq. cm. gut.	Actual/M.	Comments.
	mins.	mg.		mg.	mg.		mg.		mg.	mg.		
Isosmotic GLYCINE.	40	253.7	78.3	10.3	.21	3.37	222.7	72.9	9.3	.26	2.97	Decerebrate. 9 Results.
Isosmotic GLYCINE.	40	308.2	89.7	11.9	.24	4.11	224.9	68.0	8.8	.25	3.00	Anaesthetised. 6 Results.
Isosmotic NA GLYCINE.	40	93.6	22.0	3.2	.07	0.97	119.8	29.2	4.0	.11	1.23	Anaesthetised. 5 Results.
Isosmotic Na GLYCINE.	40	71.1	17.2	3.2	.07	0.73						Decerebrate. 1 Result.
Isosmotic Na GLYCINE (brought to pH = 7.4)	40	342.5	88.3	13.3	.27							Decerebrate. 4 Results.
Half isosmotic GLYCINE + Half isosmotic GLUCOSE.	40	116.4	67.0	4.2	.08	1.65	114.4	71.6	4.2	.12	1.56	Anaesthetised. 5 Results.
Half isosmotic GLYCINE.	40	122.2	78.4	4.2	.08	1.63	124.7	79.4	4.0	.11	1.66	Anaesthetised. 8 Results.
Half isosmotic GLYCINE.	40	118.6	77.8	3.8	.08	1.58	120.7	78.4	3.9	.11	1.64	Decerebrate. 6 Results.
Half isosmotic PHENYLALANINE.	40	238.2	72.2	8.9	.18	1.44	225.3	63.8	7.4	.21	1.36	Anaesthetised. 8 Results.
Half isosmotic PHENYLALANINE.	40	253.8	79.2	9.1	.18	1.54	210.3	64.0	6.4	.18	1.27	Decerebrate. 7 Results.

- Continued -

TABLE 28 (Contd).

ABSORPTION.
(Mean Values.)

Amino acid used.	Time of Absorption.	UPPER LOOP.					LOWER LOOP.					Comments.
		Actual.		per cm. gut.	per sq.cm. gut.	Actual/M.W.	Actual.		per cm. gut.	per sq. cm.gut.	Actual/M.W.	
	mins.	mg.	%	mg.	mg.		mg.	%	mg.	mg.		
Half isosmotic ALANINE.	40	137.2	73.3	4.8	.10	1.54	137.9	74.3	4.7	.13	1.48	Anaesthetised. 6 Results.
Half isosmotic ALANINE.	40	134.8	67.3	5.6	.11	1.51	125.4	65.5	5.3	.15	1.41	Decerebrate. 7 Results.
Half isosmotic ISOLEUCINE.	40	199.2	69.9	6.8	.14	1.52	181.1	66.5	6.3	.18	1.38	Anaesthetised. 6 Results.
Half isosmotic ISOLEUCINE.	40	166.3	59.3	5.8	.12	1.27	166.3	61.1	5.8	.16	1.27	Decerebrate. 6 Results.

(Results from all experiments not wholly free from technical fault are omitted).

means is stated.

Notation:

The notation is the same as used in the case of the rat, Section A, and is explained there. The statistical values obtained for $\bar{X}_1$, $\bar{X}_2$, $|\bar{X}_1 - \bar{X}_2|$, t and P are tabulated in Table 29.

When the difference between the means is significant, the difference is not likely to have arisen by chance.

The percentage absorption values of glycine, L(+) alanine, L(-)phenylalanine and D(-)leucine in upper and lower loops of the small intestine of the cat, given in Tables 14 to 27 are also statistically compared. In each Table the percentage absorption values in the upper loop are compared with those in the lower loop.

The notation is the same as in the rat, Section A, and is explained there. The statistical values obtained for $\bar{X}$, $S(X - \bar{X})^2$, t and P are tabulated in Table 30.

TABLE 29.

Tables compared.	$\bar{X}_1$	$\bar{X}_2$	$\lvert\bar{X}_1-\bar{X}_2\rvert$	t	P	Difference bet. the means.
14 Upper 14 Lower.	253.6	222.7	30.9	1.70	0.2-0.1	Insignificant
14 Upper 15 Upper.	253.6	308.2	54.6	4.14	0.01-0.001.	Significant
14 Lower 15 Lower.	222.7	224.9	2.2	0.93	0.4-0.3	Insignificant
15 Upper 15 Lower.	308.2	224.9	83.3	5.69	<0.001	Significant.
16 Upper.17 Upper.	93.6	71.1	22.5	0.29	0.8-0.7	Insig.
16 Upper 16 Lower.	93.6	119.8	26.2	0.94	0.4-0.3	Insig.
18 Upper 18 Lower.	342.5	257.6	84.9	1.15	0.3-0.2	Insig.
19 Upper 19 Lower.	116.4	114.4	2.0	0.23	0.9-0.8	Insig.
19 Upper 20 Upper.	116.4	122.2	5.8	0.83	0.7-0.6	Insig.
19 Lower 20 Lower.	114.4	124.7	10.3	1.85	0.1-0.05	Insig.
20 Upper 20 Lower.	122.2	124.7	2.5	0.31	0.8-0.7	Insig.
20 Upper 21 Upper.	122.2	118.6	3.6	0.39	0.7	Insig.
20 Lower 21 Lower	124.7	120.7	4.0	0.81	0.5-0.4	Insig.
21 Upper 21 Lower.	118.6	120.7	2.1	0.35	0.8-0.7	Insig.
22 Upper 23 Upper.	238.2	253.8	15.6	0.81	0.5-0.4	Insig.
22 Lower 23 Lower.	225.3	210.3	15.0	0.87	0.6-0.5	Insig.
22 Upper 22 Lower.	238.2	225.3	12.9	0.81	0.6-0.5	Insig.
23 Upper 23 Lower.	253.8	210.3	43.5	2.34	0.05-0.02	Significat
24 Upper 21 Lower.	137.2	137.9	0.7	0.50	0.7-0.6	Insig.
24 Upper 25 Upper.	137.2	134.7	2.5	0.16	0.9-0.8	Insig.
24 Lower 25 Lower	137.9	125.4	12.5	0.85	0.5-0.4	Insig.

- Continued -

TABLE 29 (Contd).

Tables compared.	$\bar{X}_1$	$\bar{X}_2$	$\lvert\bar{X}_1-\bar{X}_2\rvert$	t	p	Difference between the means.
25 Upper 25 Lower.	134.8	125.4	9.4	0.62	0.6-0.5	Insig.
26 Upper 26 Lower.	199.2	181.1	18.1	1.21	0.3-0.2	Insig.
26 Upper 27 Upper.	199.2	166.3	32.9	2.19	0.1-0.05	Barely Sig.
26 Lower 27 Lower.	181.1	166.3	14.8	0.94	0.4-0.3	Insig.
27 Upper 27 Lower.	166.3	166.3	0.0	0.00	> 0.9	Insig.

TABLE 30.

Table.	$\bar{X}$	$S(X-\bar{X})^2$	t	P	Difference between the mean and zero.
14.	5.53	1855.54	1.09	0.4-0.3	Insignificant.
15.	22.00	101.40	9.76	< 0.001	Significant.
16.	-7.15	345.50	-1.72	0.2-0.1	Insignificant
18	9.32	106.10	3.14	0.1-0.05	Barely Signifi: :cant.
19.	-4.6	316.3	-1.16	0.4-0.3	Insignificant.
20	-0.9	386.1	-0.36	0.8-0.7	Insignificant
21	-0.5	93.07	-0.28	0.8-0.7	Insignificant
22	8.9	1073.2	1.75	0.2-0.1	Insignificant
23	15.1	454.3	4.59	0.1-0.001	Significant.
24	-1.0	276.1	-0.32	0.8-0.7	Insignificant
25	2.0	1637.4	0.32	0.8-0.7	Insignificant
26	3.4	210.0	1.29	0.3-0.2	Insignificant
27	-1.8	995.8	-0.30	0.8-0.7	Insignificant

Correlation:

As in the rat, Section A, the correlation between the actual absorption values of the amino acids glycine, L(+)alanine, L(-)phenylalanine and D(-)isoleucine from upper and lower loops of the small intestine of the anaesthetised and the decerebrate cat, given in Table 28, and their apparent molal volumes is measured by the product moment method described by Chambers (1946).

The values obtained for σ_x, σ_y, $\frac{S(XY)}{n}$, $\bar{X}\bar{Y}$, r and P are tabulated below in Table 31.

TABLE 31.

Animal.	Loop	σ_x	σ_y	$\frac{S(XY)}{n}$	$\bar{X}\bar{Y}$	r
Anaesthetised	Upper	46.9	34.0	16,384.8	65,539.1	0.996
Cat	Lower.	39.4	34.0	15,532.8	62,131.4	0.984
Decerebrate.	Upper	52.2	34.0	15,936.4	63,745.8	0.920
Cat	Lower	37.3	34.0	14,416.7	57,666.8	0.972

Animal.	P	
Anaesthetised.	0.01 - 0.001.	Significant
Cat	0.01 - 0.001	Significant
Decerebrate.	0.1 - 0.05	Barely Significant
Cat	0.05 - 0.02	Significant.

A real degree of association (perfect relationship) is indicated between the actual absorption values of the amino acids studied and their apparent molal volumes. Statistical comparison of the actual absorption values(or of these values expressed as a percentage)in Tables 20 to 27 indicate that there is no difference in the rates of absorption of the half isosmotic solutions of the four amino acids in upper and in lower loops of the small intestine of the anaesthetised cat.

In the case of the decerebrate cat there is likewise no difference in the rates of absorption (or of these values expressed as a percentage) of half isosmotic glycine, alanine and isoleucine in upper and in lower loops of the small intestine. There is a difference, however, in the actual absorption values (and of these values expressed as a percentage) of half isosmotic phenylalanine in upper and in lower loops of the small intestine. The upper loop absorbs phenylalanine at a faster rate than the lower loop.

Statistical comparison of the actual absorption values of each of the half isosmotic solutions of the amino acids in upper loops and in lower loops of the small intestine of anaesthetised and decerebrate cats indicates no difference in the rates of absorption of the amino acids. Absorption is not depressed by the

anaesthetic used.

There is no difference in the rates of absorption (or of these values expressed as a percentage) from upper and lower loops of the small intestine of anaesthetised cats of glycine from a half isosmotic glycine solution and a solution containing a mixture of equal parts of half isosmotic glycine and half isosmotic glucose.

Statistical comparison of the actual absorption values (or these values expressed as a percentage) for isosmotic solutions of the sodium salt of glycine and the mixture of half isosmotic glycine and half isosmotic glucose indicates that there is no difference in the rates of absorption in upper and lower loops of the small intestine of the anaesthetised and the decerebrate cat. There is a difference, however, in the actual absorption values (and of these values expressed as a percentage) of isosmotic glycine in upper and in lower loops of the small intestine of the anaesthetised, but not the decerebrate cat. The upper loop absorbs isosmotic glycine at a faster rate than the lower loop.

Statistical comparison of the actual absorption values of isosmotic glycine (sodium salt) and the isosmotic sodium salt of glycine at pH 7.4 in upper loops and in lower loops of the small intestine of anaesthetised and decerebrate cats indicates that there is no difference

between the rates of absorption of the amino acids. Absorption is not depressed by the anaesthetic used. There is a difference, however, between the actual absorption values of isosmotic glycine in the upper loop of the small intestine of the anaesthetised cat and those in the upper loop of the small intestine of the decerebrate cat. The rate of absorption in the case of the anaesthetised cat is greater than that in the decerebrate cat. It is safe to study absorption of amino acids from isosmotic and half isosmotic solutions.

c) Discussion.

Methods of expressing Rates of Absorption:

a) Unit mucosal surface area:

Wood's figures (1944) for the relative mucosal area in jejunum and ileum are used to find the rates of absorption of glycine, L(+)alanine, L(-)phenylalanine and D(-)isoleucine per sq. cm. mucosal surface area in upper and lower loops of the small intestine. These results are seen in Tables 14 to 27 and brought together in Table 28.

b) Length of loops:

It is difficult to measure accurately a 30 cm. loop on account of vigorous movements in the small intestine. These frequently persist after death of the animal.

/Concentration:

The concentration of all solutions of alanine, phenylalanine and isoleucine used in this investigation are half isosmotic. In the case of glycine, which is very soluble in water, the rate of absorption of isosmotic and half isosmotic solutions are compared. The rate of absorption of the sodium salt of glycine, as an isosmotic solution, has also been measured.

With isosmotic and half isosmotic solutions of the amino acids under investigation the histological picture shows that there is no damage to the mucous membrane of the small intestine of the cat. However, desquamation of the epithelial cells of the villi is apparent when the sodium salt of glycine remains in the lumen of the gut for any length of time - Figs. 18 and 19. This may be due in part at least to the alkaline nature of the solution (pH = 10.0).

Cats under Urethane Anaesthesia:

Relative rates of absorption:

A comparison of the results from half isosmotic solutions of the amino acids in Tables 20, 22, 24, 26 and 28 shows that L (-) phenylalanine has the greatest rate of absorption whether the rate is expressed in mg. phenylalanine absorbed per sq. cm. gut or mg. phenylalanine absorbed per sq. cm. mucosal surface area. D (-) Isoleucine is

absorbed more slowly than L(-) phenylanine and L(+) alanine and glycine still more slowly. Glycine has the slowest absorption rate of all four amino acids.

Isoleucine:

The rate of absorption of D(-) isoleucine in the upper loop of the small intestine of the cat is slightly more than that in the lower loop if the absorption rates are expressed in mg. per cm. gut. The rate of absorption in the upper loop is 6.8 mg. isoleucine absorbed per cm. gut and 6.4 mg. per cm. gut in the lower loop. The time of absorption in both cases is 40 minutes. If, however, the absorption rate is expressed per unit mucosal surface area there is a definite difference in the absorption rates in upper and lower loops of the small intestine. The lower loop absorbs isoleucine at a greater rate than the upper loop, the lower loop absorbing 0.18 mg. per sq. cm. mucosal surface area while the upper loop absorbs 0.14 mg. per sq. cm. mucosal surface area in 40 minutes. These results are not statistically different.

Phenylalanine:

In the case of L(-) phenylalanine, the rate of absorption in the upper loop of the small intestine of the cat is greater than that in the lower loop if the rates are expressed in mg. per cm. gut - the average absorption rate of phenylalanine is 8.9 mg. per cm. gut

in the upper loop and 7.4 mg. per cm. gut in the lower loop during an absorption period of 40 minutes. When the absorption rate is expressed in mg. phenylalanine absorbed per unit mucosal surface area the greater rate of absorption in the lower loop becomes more marked - the upper loop absorbing 0.18 mg. phenylalanine per sq. cm. mucosal surface area in 40 minutes while the lower loop absorbs 0.21 mg. phenylalanine per sq. cm. mucosal surface area in the same time. These results are not statistically different.

Alanine:

The rates of absorption of L(+)alanine in upper and lower loops of the small intestine of the cat appear to be approximately equal - the average absorption rate of alanine is 4.79 mg. per cm. gut in the upper loop and 4.71 mg. per cm. gut in the lower loop during an absorption period of 40 minutes. On the other hand, when the absorption rates are expressed in mg. alanine absorbed per unit surface area the general trend of more rapid absorption in the lower loop is apparent. The lower loop absorbs 0.13 mg. alanine per sq. cm. mucosal surface area in 40 minutes while the upper loop absorbs 0.10 mg. alanine per sq. cm. mucosal surface area in the same time. These results are not statistically different.

Glycine:

Similar variations occur in the rates of

absorption of glycine in upper and lower loops of the small intestine of the cat. About equal absorption rates take place in upper and lower loops of the small intestine when the rates are expressed in mg. per cm. gut - 4.2 mg. and 4.0 mg. glycine absorbed per cm. gut respectively. When the absorption rates are expressed in mg. glycine absorbed per unit surface area per 40 minutes greater absorption appears to take place in the lower loop - 0.11 mg. glycine per sq. cm. mucosal surface area in 40 minutes while the upper loop absorbs 0.08 mg. glycine per sq. cm. mucosal surface area in the same time. These results are not statistically different.

Milli molar rates of absorption:

A comparison between the milli molar rates of absorption in upper and lower loops of the small intestine of the amino acids studied shows that glycine has the greatest milli molar rate of absorption, namely, 1.63 in the upper loop, and 1.66 in the lower loop. Alanine comes next with milli molar rates of absorption of 1.54 and 1.48 in upper and lower loops respectively. Isoleucine has even lower milli molar absorption rates - 1.52 and 1.38, and phenylalanine the smallest with 1.44 and 1.36 in upper and lower loops respectively.

The milli molar absorption rate of glycine in the lower loop of the small intestine of the cat is slightly higher than that in the upper loop. In the case of

alanine, isoleucine and phenylalanine the milli molar absorption rate is higher in the upper loop than it is in the lower loop.

A statistical comparison made between the actual absorption rates of half isosmotic solutions of D (-) isoleucine, L (-) phenylalanine, L(+) alanine and glycine in upper and lower loops of the small intestine of the anaesthetised cat shows that the difference between the means is insignificant. A similar comparison between the actual absorption rates expressed as a percentage shows that the difference between the mean and zero is also insignificant.

Decerebrate Cats:

Relative rates of absorption:

A study of the results in Tables 21, 23, 25, 27 and 28 from half isosmotic solutions of the amino acids shows that L (-) phenylalanine has the greatest rate of absorption whether expressed in mg. phenylalanine absorbed per cm. gut or mg. phenylalanine absorbed per sq. cm. mucosal surface area. D (-) isoleucine is absorbed at a lower rate while L (+) alanine and glycine are absorbed still more slowly - glycine being the slowest of all four amino acids.

Isoleucine:

The rates of absorption of D (-) isoleucine in

upper and lower loops of the small intestine of the decerebrate cat are approximately equal and are not statistically different. The average absorption rate of isoleucine is 5.77 mg. per cm. gut in both upper and lower loops during an absorption period of 40 minutes. When the absorption rates are expressed in mg. isoleucine absorbed per sq. cm. mucosal surface area there is a definite difference in the rates of absorption in upper and lower loops of the small intestine. The lower loop absorbs isoleucine at a greater rate than the upper loop, the lower loop absorbing 0.16 mg. isoleucine per unit mucosal surface area while the upper loop absorbs 0.12 mg. isoleucine per unit mucosal surface area in 40 minutes.

Phenylalanine:

In the case of L (-) phenylalanine, the rate of absorption in the upper loop is more than that in the lower loop and the absorption values are statistically different. When the rates of absorption are expressed in mg. per cm. gut the rate of absorption in the upper loop of the small intestine of the decerebrate cat is 9.06 mg. phenylalanine absorbed per cm. gut and 6.40 mg. phenylalanine absorbed per cm. gut in the lower loop. The time of absorption in both cases is 40 minutes. However, if the absorption rates are expressed in mg. phenylalanine absorbed per unit mucosal surface area

there is very little difference between the rates in the upper and lower loops. Both loops absorb phenylalanine at the rate of 0.18 mg. per sq. cm. mucosal surface area in 40 minutes.

Alanine:

Similar variations occur in the rates of absorption of L(+) alanine in upper and lower loops of the small intestine of the decerebrate cat. When the absorption rate is expressed in mg. per cm. gut slightly greater absorption takes place in the upper loop than in the lower loop - 5.5 mg. and 5.3 mg. alanine per cm. gut respectively. When the absorption rates are expressed in mg. alanine absorbed per unit mucosal surface area per 40 minutes greater absorption again appears to take place in the lower loop - 0.15 mg. alanine per unit mucosal surface area in 40 minutes while the upper loop absorbs 0.11 mg. alanine per unit mucosal surface area in the same time. These results are not statistically different.

Glycine:

When the absorption rates of glycine in upper and lower loops of the small intestine of the decerebrate cat are expressed in mg. per cm. gut slightly greater absorption takes place in the lower loop. The absorption rate of glycine in the upper loop is 3.84 mg. per cm. gut and in the lower loop it is 3.88 mg. per cm. gut.

If the rates of absorption are expressed in mg. glycine absorbed per unit mucosal surface area more rapid absorption in the lower loop is apparent. The upper loop absorbs 0.08 mg. glycine per sq. cm. mucosal surface area in 40 minutes while the lower loop absorbs 0.11 mg. glycine per sq. cm. mucosal surface area in the same time. These results are not statistically different.

Milli molar rates of absorption:

From a consideration of the results for upper loops of the small intestine of the decerebrate cat in Tables 21, 23, 25, 27 and 28 it is clear that glycine has the greatest milli molar rate of absorption, namely 1.58. L(-)phenylalanine is next with 1.54 followed by L(+)alanine, 1.51. D(-)isoleucine has the lowest milli molar rate of absorption of the four amino acids, 1.27.

In the lower loops glycine again has the highest milli molar absorption rate - 1.64, and isoleucine the lowest - 1.27. In contrast to the upper loop, however, alanine has a greater milli molar absorption rate - 1.41, than phenylalanine - 1.27.

The milli molar rate of absorption of glycine is slightly higher in the lower loop than in the upper loop. In the case of alanine and phenylalanine it is the upper loop that exhibits a higher milli molar rate of absorption. Isoleucine has identical milli molar absorption rates in

both upper and lower loops.

Statistics:

A statistical comparison made between the actual rates of absorption of half isosmotic solutions of D (-) isoleucine, L (-) phenylalanine, and L (+) alanine and glycine in upper and lower loops of the small intestine of the decerebrate cat shows that the difference between the means is insignificant except in the case of L (-) phenylalanine where the difference is significant. A similar comparison between the actual absorption rates expressed as a percentage shows the difference between the mean and zero to be significant in the case of L (-) phenylalanine but insignificant for the other three amino acids.

Absorption of half isosmotic solutions of amino acids from the small intestine of Decerebrate and of Anaesthetised Cats:

Relative rates of absorption:

A comparison of Tables 20 to 27 and 28 shows that the rate of absorption of glycine and of D (-) isoleucine in upper and lower loops of the small intestine whether expressed in mg. amino acid absorbed per cm. gut or mg. amino acid absorbed per unit mucosal surface area, is greater in the case of the cat under urethane anaesthesia than in the decerebrate animal. But L (+) alanine, on the other hand, is absorbed more rapidly in the upper and lower loops of the small intestine of the decerebrate cat

than it is in the small intestine of the anaesthetised cat. The upper loop of the small intestine of the decerebrate cat shows greater absorption of L(-) phenylalanine than that of the anaesthetised cat whereas the converse is found in the lower loop.

Milli molar rates of absorption:

Tables 20 to 27 and 28, show that the milli molar rate of absorption of glycine, L(+) alanine and D(-) isoleucine is greater in the upper and lower loops of the small intestine of the anaesthetised cat than it is in the small intestine of the decerebrate cat. The milli molar rate of absorption of L(-) phenylalanine is also greatest in the lower loop of the small intestine of the cat under urethane anaesthesia. In the upper loop of the small intestine of the decerebrate cat, however, the milli molar absorption rate of L(-) phenylalanine is greater than that in the upper loop of the small intestine of the anaesthetised cat.

It will be seen, therefore, that on the whole the rates of absorption of the four amino acids from the small intestine of the decerebrate cat are slightly lower than the absorption rates of these amino acids from the small intestine of the anaesthetised cat, whether these rates of absorption are expressed in mg. per unit length, mg. per unit mucosal surface area or as milli molar absorption rates.

A statistical comparison made between the actual absorption rates of the half isosmotic amino acids from upper loops of the small intestine of decerebrate and anaesthetised cats shows thedifference between the means to be insignificant in the caseof glycine, L (+) alanine, and L (-)phenylalanine, and barely significant in the case of D(-)isoleucine. When the actual absorption rates from lower loops of the small intestine of decerebrate and anaesthetised cats are statistically compared, the difference between the means is insignificant. (Tables 29 and 30).

Graphs:

A study of the graphs in Fig. 20 shows a general tendency for the rate of absorption of the amino acids in mg. per 40 minutes in both upper and lower loops of the decerebrate and the anaesthetised cat to rise as the molecular weight increases. Similarly, in Fig. 21 the rate of absorption of the amino acids in mg. per 40 minutes in both upper and lower loops rises as the apparent molal volume increases. The correlation coefficients of the relationship are:-

	Loops	r	P	Relationship.
Decerebrate Cat.	Upper	0.920	0.01-0.05	Barely significant
	Lower	0.972	0.05-0.02	Significant.
Anaesthetised Cat.	Upper	0.996	0.01-0.001	Significant.
	Lower	0.984	0.01-0.001	Significant.

The shapes of the graphs in Fig. 20 show a general correspondence to the shapes of the graphs in Fig. 21. After the common point on the graphs for the decerebrate animal there is a sharp increase in the gradient of the graph for absorption in the upper loop of the small intestine.

The graphs in Fig. 22 show that as the milli molar rate of absorption decreases the molecular weight increases. Similarly, the graphs in Fig. 23 for decerebrate and for anaesthetised cats indicate that as the milli molar absorption rate decreases the apparent molal volume increases. Again, the shapes f the graphs in Fig. 22 show a general correspondence to the shapes of the graphs in Fig. 23. There is the same tendency for a sharp increase in the gradient of the graph for absorption in the upper loop of the small intestine of the decerebrate cat after the point in common.

This sharp increase in the gradient caused by the absorption rate of phenylalanine agrees well with the data for the chick plotted by Kratzer (1944) in Fig.10. There is good correspondence between the shape of Kratzer's graph and the graphs given in Figs.23. Kratzer studied the absorption rate of L-leucine (sodium salt) in the intestinal tract of the chick whereas D-isoleucine is used in the present investigation. However, as the

apparent molal volumes of isomers of the amino acids are the same (Cohn and Edsall, 1943), the shape of Kratzer's graph and of the graph above may be reasonably compared.

Cats and Rats under Urethane Anaesthesia.

Rates of absorption per unit length and per unit mucosal surface area:

A true comparison between rates of absorption per unit length in the cat and in the rat is not possible since the diameter of the small intestine of the cat is so much greater than in the rat. However, one can compare the relative rates of absorption per sq. cm. mucosal surface area in both cat and rat as the mucosal surface area is independent of the diameter of the gut.

Table 32 shows that the small intestine of the cat (especially in the upper regions) absorbs glycine, alanine, phenylalanine and isoleucine at a faster rate than the small intestine of the rat even allowing for a greater degree of villous development in the small intestine of the cat.

A comparison of the relative rates of absorption of half isosmotic solutions of the amino acids from upper and lower loops of the small intestine of the cat and rat under urethane anaesthesia is possible when the average results of Tables 8 and 28 are examined. In the rat the relative

TABLE 32.

Amino acid.	UPPER LOOP.				LOWER LOOP.			
	Absorption per sq.cm.gut				Absorption per sq. cm. gut.			
	Rat.	Cat.	Difference.		Rat.	Cat.	Difference.	
	mg.	mg.	mg.	%	mg.	mg.	mg.	%
Glycine.	.0747	.0845	+.0098	11.7	.1107	.1136	+.0029	2.6
Alanine	.0712	.0969	+.0257	36.1	.1025	.1326	+.0301	29.3
Phenylalanine	.1095	.1780	+.0685	62.5	.1928	.2097	+.0169	8.7
Isoleucine.	.0837	.1374	+.0537	64.1	.1381	.1788	+.0407	29.4

rates of absorption in both upper and lower loops, whether expressed as mg. amino acid absorbed per cm. gut or mg. amino acid absorbed per sq. cm. mucosal surface area, are in the order L(-)phenylalanine > D(-)isoleucine > glycine > L(+) alanine. In the upper and lower loops of the small intestine of the cat the order of the rates is L(-)phenylalanine > D(-) isoleucine > L(+)alanine > glycine. In the anaesthetised rat glycine is absorbed at a faster rate than L(+)alanine whereas in the anaesthetised cat the reverse occurs. In both rat and cat L(-)phenylalanine has the greatest rate of absorption. D(-)isoleucine is absorbed at a slightly lower rate than L(-)phenylalanine.

Milli molar absorption rates:

When the milli molar rates of absorption of half isosmotic solutions of the amino acids from the small intestine of anaesthetised rats and cats are compared, a difference in the above order is observed. In the upper loop of the small intestine of the cat the order of the milli rates of absorption is glycine > L(+)alanine > D(-) isoleucine > L(-)phenylalanine whereas in the rat the order is glycine > L(+)alanine > L(-)phenylalanine > D(-)isoleucine. In the lower loop of the small intestine of the cat the order of the milli molar absorption rates is the same as in the upper loop, namely glycine > L(+)alanine > D(-)isoleucine > L(-)phenylalanine; the order in the lower loop of the small intestine of the rat is glycine > L(-)phenylalanine > L(+)alanine > D(-)isoleucine.

In both the anaesthetised cat and rat the milli molar rate of absorption of glycine is the highest of all the amino acids studied. In the cat the order is the same in both upper and lower loops - glycine fastest and L(-) phenylalanine slowest, whereas in the rat glycine is absorbed fastest and D(-)isoleucine slowest.

Isosmotic Glycine: Anaesthetised Cats.

Rates of Absorption:

A comparison of the results from isosmotic solutions of amino acids in Tables 15, 16 and 28 shows that glycine has a much greater absorption rate than its sodium salt whether the rate is expressed as mg. amino acid absorbed per cm. gut or per unit mucosal surface area.

Glycine:

The rate of absorption of isosmotic glycine in the upper loop of the small intestine of the cat is greater than that in the lower loop. The absorption values are significantly different. If the rate is expressed in mg. absorbed per cm. gut the absorption rate in the upper loop is 11.9 mg. glycine per cm. gut and in the lower loop it is 8.8 mg. The period of absorption is, in both cases, 40 minutes. If, however, the absorption rate is expressed per unit surface area there is a difference, the lower loop absorbs at a slightly greater rate than does the upper loop of the small intestine. The lower loop absorbs 0.25 mg. glycine per sq. cm. mucosal surface area in 40 minutes while

the upper loop absorbs 0.24 mg. glycine per sq. cm. mucosal surface area in the same time.

Sodium salt of glycine:

The rate of absorption of the sodium salt of glycine is greater in the lower loop than in the upper loop of the small intestine - the average absorption rate per cm. gut in the lower loop is 4.0 mg. and in the upper loop 3.2 mg. per cm. gut. When the absorption rates are expressed as mg. absorbed per unit surface area the lower loop absorbs 0.11 mg. per sq. cm. mucosal surface area per 40 minutes and the upper loop 0.07 mg. per sq. cm. mucosal surface area in the same time. These values are not s-tatistically different.

Milli molar rates of absorption:

Tables 15, 16 and 28 show that isosmotic glycine has a much greater milli molar absorption rate than the isosmotic sodium salt of glycine in upper and lower loops of the small intestine of the anaesthetised cat. The milli molar absorption rate of glycine in the upper loop of the small intestine of the cat is 4.1, and in the lower loop, 3.0. The milli molar rates of absorption of the sodium salt of glycine in upper and lower loops of the small intestine of the cat are 0.97 and 1.23 respectively.

Comparison with half isosmotic glycine:

A comparison of the results in Tables 15, 16,

29 and 28 clearly shows that in upper and lower loops of the small intestine of the anaesthetised cat half isosmotic glycine, although absorbed at a much slower rate than isosmotic glycine, has a greater absorption rate than the isosmotic sodium salt of glycine. This is the case whether expressed as the milli molar absorption rate, as mg. amino acid absorbed per cm. gut or mg. amino acid absorbed per sq. cm. mucosal surface area.

The rates of absorption of isosmotic glycine, half isosmotic glycine and the isosmotic sodium salt of glycine in the small intestine of the cat (in mg. absorbed per cm. gut) are:-

	UPPER LOOP.	LOWER LOOP.	DIFFERENCE.	
	mg.	mg.	mg.	%
Isosmotic glycine.	11.90	8.78	+3.12	34.7
Half isosmotic glycine.	4.18	4.03	+0.15	3.7
Isosmotic glycine(Na)	3.24	4.00	-0.76	23.4

The absorption rates of isosmotic glycine, half isosmotic glycine and the isosmotic sodium salt of glycine, expressed in mg. per unit surface area are:-

	UPPER LOOP.	LOWER LOOP.	DIFFERENCE.	
	mg.	mg.	mg.	%
Isosmotic glycine.	0.24	0.25	-0.01	4.1
Half isosmotic glycine.	0.09	0.11	-0.02	22.2
Isosmotic glycine(Na)	0.07	0.11	-0.04	57.1

The milli molar rates of isosmotic glycine, half isosmotic glycine and the isosmotic sodium salt of glycine are:-

	UPPER LOOP.	LOWER LOOP.	DIFFERENCE.	
	mg.	mg.	mg.	%
Isosmotic glycine.	4.11	3.00	+1.11	37.0%
Half isosmotic glycine.	1.63	1.66	-0.03	1.8%
Isosmotic glycine(Na).	0.97	1.24	-0.27	27.8%

These results are in sharp contrast to the finding in the rat, Section A, where there was very little difference in the absorption rates per hour of isosmotic and half isosmotic glycine from upper and lower loops of the small intestine. It is recalled that Cori (1926-27) found that the rate of absorption of glycine, in the rat, was within wide limits independent of the absolute amount and concentration. Kratzer (1944), working with chicks, also found that glycine gave practically the same absorption rate at a high level of administration as it did at a lower level.

Statistics:

A statistical comparison made between the actual rates of absorption of isosmotic glycine in upper and lower loops of the small intestine of the anaesthetised cat shows that the difference between the means is significant (Table 29). A similar comparison between the actual absorption rates expressed as a percentage shows the difference between the mean and zero to be likewise significant (Table 30).

A statistical comparison between the actual rates of absorption of the isosmotic sodium salt of glycine in upper and lower loops of the small intestine of the anaesthetised cat shows the difference between the means to be insignificant. A similar comparison between these rates expressed as a percentage shows the difference between the mean and zero is likewise insignificant.

Isosmotic Glycine : Decerebrate Cats.

Rates of Absorption:

A study of the rates of absorption of isosmotic solutions of glycine and its sodium salt in upper loops of the small intestine of the decerebrate cat in Tables 14, 17 and 28 makes it clear that glycine is absorbed at a much greater rate than the sodium salt however the rates are expressed. The rates of absorption of isosmotic glycine and the isosmotic sodium salt of glycine in the upper loop expressed as mg. absorbed per unit length are 10.3mg. and 3.2 mg. respectively. Expressed in terms of mg. absorbed per unit mucosal surface area the values are 0.21 mg. and 0.07 mg. respectively.

Only one decerebrate animal was used when studying the absorption rate of the isosmotic sodium salt - the absorption rate of the sodium salt being measured in an upper loop of the small intestine while the absorption rate of isosmotic glycine was measured in the lower loop for

direct comparison. The absorption rate in the lower loop was very much greater than in the upper loop, the absorption rate of the glycine falling in line with other similar measurements. At this stage it was decided to abandon measurements of absorption rates of sodium salts of the amino acids from the small intestine of the cat.

Milli molar absorption rates:

Tables 14, 17 and 28 show clearly that isosmotic glycine has a much greater milli molar absorption rate than the isosmotic sodium salt of glycine from the upper loop of the small intestine of the decerebrate cat. The milli molar rate of absorption of glycine is 3.37 and that of its sodium salt, 0.73.

Effect of pH:

The pH of the isosmotic sodium salt of glycine is 10.0 whereas that of an isosmotic solution of glycine is 7.4. The alkalinity of the sodium salt is doubtless the cause of the epithelial desquamation which occurs (Figs. 18 and 19). It was noticed, when washing the mucosa of the loops during recovery of the residual sodium salt of glycine, that the epithelium of the loop was reddened and looked irritated. When the pH of the solution is lowered to 7.4 by addition of N/1 hydrochloric acid the absorption of this solution approaches that of isosmotic glycine (Tables 18 and 28). It is likely that the hydrochloric acid

has converted the sodium salt back to glycine.

Statistics:

A statistical comparison made between the actual rates of absorption of isosmotic glycine in upper and lower loops of the small intestine of the decerebrate cat shows that the difference between the means is insignificant (Table 29). A similar comparison between the actual absorption rates expressed as a percentage shows the difference between the mean and zero to be likewise insignificant (Table 30).

Absorption of isosmotic solutions from the small intestine of anaesthetised and decerebrate cats.

Relative rates of absorption:

A comparison of Tables 14 to 17 and 28 shows that the rate of absorption of glycine and its sodium salt in upper loops of the small intestine, whether expressed in mg. absorption per cm. gut or mg. absorption per unit mucosal surface area, is greater in the case of the cat under urethane anaesthesia than in the decerebrate animal. In the lower loop, on the other hand, the decerebrate animal absorbs glycine more rapidly than one under urethane anaesthesia.

Milli molar rates of absorption:

Tables 14 to 17 and 28 show that the milli molar absorption rate of glycine, in upper and lower loops

of the small intestine of the anaesthetised cat, is greater than in loops of the small intestine of the decerebrate animal.

Statistics:

A statistical comparison made between the actual rates of absorption of the isosmotic amino acids from upper loops of the small intestine of anaesthetised and decerebrate cats shows the difference between the means to be significant in the case of glycine and insignificant in the case of the sodium salt of glycine. When the absorption rates from lower loops of the small intestine of anaesthetised and decerebrate cats are statistically compared the difference between the means is insignificant.

Glycine/Glucose mixture:

In Table 19 are given the absorption values of a mixture of equal parts of half isosmotic glycine and half isosmotic glucose from the small intestine of the anaesthetised cat. A comparison of the results in Table 19 with those for half isosmotic glycine in the anaesthetised cat (Table 20) shows that in the upper loop of the small intestine the glycine in the glycine/glucose mixture is absorbed at a slower rate than the half isosmotic glycine. The absorption rate of glycine in the upper loop of the small intestine of the cat is 4.18 mg.per cm. gut or 0.08 mg. per sq. cm. mucosal surface area. Corresponding figures

for the glycine in the mixture are slightly less - 4.16 mg. per cm. gut or 0.08 mg. per sq. cm. mucosal surface area. The milli molar absorption rate, too, is lower in the case of the glycine in the mixture - 1.55, compared with 1.63 for glycine. The results are not statistically different.

In the lower loop of the small intestine of the anaesthetised cat the absorption values for the glycine in the mixture are slightly greater than for glycine except where the milli molar absorption rate is expressed. The absorption rate of glycine in the lower loop of the small intestine of the cat is 4.03 mg. per cm. gut or 0.11 mg. per sq.cm.mucosal surface area. Corresponding figures for the glycine in the mixture are slightly greater - 4.17 mg. per cm. gut or 0.12 mg. per sq. cm. mucosal surface area. When the milli molar absorption rates are expressed, however, the trend of slightly slower absorption of glycine in the mixture is apparent - 1.55 for glycine in the mixture and 1.66 for glycine.

In Table 20 the rates of absorption of glycine in the mixture from upper and lower loops of the small intestine of the anaesthetised cat are statistically compared with those of glycine. The difference between the means when the upper loops in Table 19 are compared with the upper loops in Table 20 is insignificant. The difference between the means when the lower loops in

Table 19 are compared with the lower loops in Table 20 is also insignificant.

These findings illustrate the phenomenon called mutual inhibition of absorption and confirm the work of Cori (1926-27). Cori fed rats a mixture of glycine and glucose in molecular proportion and found only 0.10 g. glucose and 0.04 g. glycine were absorbed per 100g. weight of rat per hour. When fed alone, the absorption rates were 0.18 g. glucose and 0.05 g. glycine.

IV. SUMMARY.

The relative rates of absorption of the sodium salt of glycine, of glycine, L(+)alanine, L(-)phenylalanine and D(-)isoleucine from upper and lower loops of the small intestine of the anaesthetised cat and the decerebrate cat were determined. These absorption rates were expressed as the milli molar rate of absorption as well as in terms of mg. amino acid absorbed per cm. gut, and mg. amino acid absorbed per sq. cm. mucosal surface area.

When the absorption rates in upper and lower loops of the small intestine of the anaesthetised cat were expressed in terms of unit gut length, glycine, alanine, isoleucine and phenylalanine were all absorbed more rapidly in the upper loop than in the lower loop. However, when the rates of absorption were expressed in terms of

unit mucosal surface area, glycine, alanine, isoleucine, and phenylalanine appeared to be more rapidly absorbed in the lower loop than in the upper loop.

When the absorption rates in upper and lower loops of the small intestine of the decerebrate cat were expressed in terms of unit gut length, alanine and phenylalanine, but not glycine, were absorbed more rapidly in the upper loop than in the lower loop. Isoleucine was absorbed at the same rate in both upper and lower loops. When the rates of absorption were expressed in terms of unit mucosal surface area glycine, alanine, isoleucine and phenylalanine appeared to be more rapidly absorbed in the lower loop than in the upper loop.

The milli molar absorption rates of glycine, alanine, isoleucine and phenylalanine are greater in the upper loop of the small intestine of anaesthetised and decerebrate cats than in the lower loop.

Data for the milli molar absorption rates per 40 minutes and the absorption rates in mg. per 40 minutes of the amino acids from upper and lower loops of the small intestine of anaesthetised and decerebrate cats were plotted against molecular weight and apparent molal volume respectively and compared. As the molecular weight increased the absorption rate in mg. per 40 minutes of the amino acids increased and the milli molar absorption

rate per 40 minutes of the amino acids increased. As the milli molar absorption rate of the amino acids increased, the apparent molal volume and the molecular weight decreased.

In the rat, isosmotic and half isosmotic glycine were absorbed at the same rate, but in the cat isosmotic glycine was absorbed much more rapidly than half isosmotic glycine.

The small intestine of the cat absorbs glycine, alanine, phenylalanine and isoleucine at a faster rate than the small intestine of the rat even allowing for a greater degree of villous development in the small intestine of the cat.

From a mixture of glycine and glucose in equal parts, glycine was absorbed slightly less rapidly than it would be were it administered by itself in the same concentration.

The sodium salt of glycine was absorbed exceedingly slowly.

The absorption rates of amino acids from the small intestine of anaesthetised cats were slightly higher than those from the small intestine of decerebrate cats. However, a statistical comparison made between these values from anaesthetised and decerebrate cats showed the difference between the means to be insignificant in all cases except isosmotic glycine in upper loops of the small intestine

where the difference was significant.

A statistical analysis was made of all results.

---oOo---

GENERAL DISCUSSION.

GENERAL DISCUSSION.

For many years it has been generally accepted that intestinal absorption of certain dissolved substances is more than a mere diffusion. This belief is mainly based on the work of Nagano (1902) and of Cori (1925). Opinion seems to be divided as to whether amino acids are absorbed preferentially from the small intestine by a cellular mechanism or by a process of simple diffusion.

Diffusion coefficients:

The purely physico-chemical aspect of the problem has been studied by Mehl and Schmidt (1937) using a sintered glass diffusion cell. The cell was calibrated with 0.1N potassium chloride, a substance for which the diffusion coefficient had already been found by another method. The diffusion coefficients of glycine and other amino acids were determined over a fairly large range of concentrations, and at several temperatures.

The relation which has been most generally useful in the calculation of results from diffusion experiments is Fick's law (1855): $ds/dt = -AD(\partial c/dx)$ where ds is the quantity of solute passing through a cross section of area, A, and of thickness, dx, in the time, dt, where dc is the change of the concentration over the distance,

dx, and D is the diffusion coefficient.

It has become evident that the diffusion coefficient, D, may vary somewhat with the concentration.

Relation between diffusion coefficients and molecular size:

Many attempts have been made to relate the diffusion coefficient to the molecular weight. These attempts are based on the observation by Hüfner (1897), that the diffusion of gases in water could be roughly expressed by the equation:- $D\sqrt{M} = \text{Constant}$ where D is the diffusion constant and M is the molecular weight. This "constant" may vary, however, by as much as 25%.

This relationship might hold with respect to the diffusion of gases through other gases. With regard to solutions, the frictional forces outweigh those involved in imparting kinetic energy to the molecules so there is no reason to expect the mass of the molecule to be a factor in the rate of diffusion.

It seemed more reasonable to Mehl and Schmidt (1937) to suppose that the diffusion coefficient is a function of the volume and shape of the molecule, the viscosity of the medium, and the temperature. According to Cohn and Edsall (1943) "In a general way, the diffusion coefficient and the molecular weight or

molecular volume stand in the expected relation. The trend of decreasing diffusion coefficients with increasing molecular size is fairly regular, though there are some inversions in order .....it may be reasonably expected that other factors, such as the shape of the molecule, will influence the diffusion coefficient. At present, however, there is no satisfact: :ory method for treating this problem."

Schmengler, (1933), has compared the rates of penetration through collodion membranes of several amino acids with the rates of penetration of glycerine, erythritol, arabinose, mannitol and lactamide (Table 33). He found that the rates of penetration of the amino acids were lower, in comparison with the other compounds, than the molal volumes, as calculated from the molecular refractions, would lead one to expect. Although the use of molecular refractions as a measure of the molal volumes may not be altogether justifiable, the trend of the results is the same when the apparent molal volumes obtained from density results are considered. From density measurements the apparent molal volume of glycerine at 25°C (Internation: :al Critical Tables 1929) is about 58 c.c. per mole. Although the apparent molal volume of glycine is only

44.5 c.c. it penetrates the collodion membrane at a slower rate than glycerine. Schmengler attributed this behaviour to hydration of the amino acid because of the charges borne by the zwitterion. He found that the rates of penetration of alanine and arabinose were equal and that alanine penetrated more rapidly than asparagine. The diffusion coefficient of a-rabinose at 20°C and infinite dilution is 0.54 - 0.58 sq. cm. per day (Öholm, 1910). Mehl and Schmidt (1937) found the diffusion coefficients of alanine and asparagine at 20°C and infinite dilution to be 0.63 and 0.58 sq. cm. per day. Thus, from the diffusion coefficients, the rate of penetration of arabinose should have been equal to that of asparagine rather than to that of alanine. It is curious that the values of the diffusion coefficients fall in the order of the reciprocals of the molecular refractions given by Schmengler (alanine, 21.01; asparagine, 29.06; arabinose, 31.40). These results suggest some specific influence of the collodion membrane, and point to the danger of ignoring such effects, as well as the need to consider, in permeability studies, factors other than the diffusion coefficient (Table 33).

Physiological applications:

Höber and Höber (1937), in experiments with loops of the small intestine of rats under nembutal

TABLE 33.

Amino acid.	MV(Calc).	Penetration ratio	Substance compared.	MV.
Glycine.	76.5	<	Glycerol	87.8
		>	Erythritol	130.2
∝ Alanine.	98.5	<	Glycerol	87.8
		<	Erythritol	130.2
		<	Lactamide	98.5
		=	Arabinose	153.4
Leucine.	164	<	Arabinose	153.4
		=	Mannitol	189.2
Lysine.	177	<	Arabinose.	153.4
		<	Mannitol	189.2
Asparagine.	134.7	<	Arabinose	153.4
		<	Mannitol	189.2
Aspartic acid.	129.5	<	Arabinose	153.4
		<	Mannitol	189.2

-----oOo-----

anaesthesia, compared the absorption rate of several amino acids with those of polyhydric alcohols and aliphatic amides of known molal volumes. Schmenglor had found that amino acids diffused more slowly through collodion membranes than other substances of corresponding molecular volume. This slowness in diffusion of amino acids was explained on the basis of their ampholyte character, according to which in aqueous solution they are entirely present as zwitterions, i.e. as strong dipoles enlarging their molecular volume by the formation of a shell of water molecules. Consequently a porous membrane such as the intestinal wall could be expected to be passed comparatively slowly. Höber and Höber have shown that the reverse is true. The table below (Table 34) summarises the results they obtained.

With the polyhydric alcohols the absorption rate falls off with rising molecular volume. The absorption rate of aliphatic amides is analogous to that of the polyhydric alcohols of corresponding molecular volume. In the case of polyhydric alcohols absorption resembles diffusion through a sievelike membrane, the maximum pore diameter of which is the same as the external dimensions of the mannitol molecule, so that mannitol (C_6) is scarcely absorbed at all, but adonitol (C_5) and erythritol (C_4) are comparatively rapidly absorbed. But with the amides the effect of lipoid solubility is of marked importance.

TABLE 34.

Table showing whether the absorption of equimolar solutions of amino acids is faster or slower than acid amides, polyhydric alcohols, or sugar.

Amino acid.	MV(Calc).	MV(eff).	Absorption ratio	Substance compared.	MV.
Glycine.	78	110	<	Acetamide	69
			>	Lactamide.	98
			>	Malonamide	104
			>	Succinamide	126
DL∝ alanine	98.5	150	=	Glycerol	87.8
			>	Succinamide	126
			>	Erythritol	130
DL-asparagine	130	170	>	Malonamide	104
			>	Succinamide	126
			>	Erythritol	130
			>	Xylose	153
DL ∝ leucine.	164	190	=	Malonamide	104

Thus butyramide, with a molecular volume of 113, should penetrate a sieve more slowly than acetamide (molecular volume 69), but in fact butyramide penetrates rather more rapidly than acetamide. Höber and Höber explain this difference on the grounds that there are special affinities between the pore walls and the solute. They claim to show that the preferential absorption of the polyhydric alcohols is by no means indicative of a preferential absorption as brought about by some special cellular activity and that such a preferential absorption of alcohols is a diffusion process as well as that of the amides.

Höber and Orskov (1933) postulate that the cell surfaces differ from each other by the composition of their building material. For example, their lipoids being more or less acidic might be a good solvent for amides. Such behaviour can be shown by a mixture of oil and oleic acid which is a better solvent for acid amides than is neutral oil (Watzadse, 1929; Collander and Barlündo, 1933). Similarly, it seems possible to find lipoid solvents showing preferential solubility to polyhydric alcohols (Fleischmann, 1928). Finally, instead of some real dissolving power the structure of the cell surface might show particular adsorption affinities to different substances (Höber, 1936b).

Höber and Höber also considered whether the preferential absorption of the amino acids resembles that of the polyhydric alcohols and that of the acid amides, i.e., whether the diffusion of the amino acid is accelerated by adsorptive properties exerted by a porous sievelike surface membrane so that the concentration gradient inside the pores would be raised. The concept that the intestinal membrane resembles a molecular sieve is not a new one and was proposed as early as 1890 by Waymouth Reid. By varying the concentration of the amino acids Höber and Höber found that the percentage rate of absorption was not constant but fell off with rising concentration. From this observation they argued that amino acids resembled the physiological sugars (glucose, galactose) and that their absorption was not a process of mere diffusion but was speeded up by some unknown preferential factor more apparent with lower than with higher concentrations. In this respect, the amino acids differed from the polyhydric alcohols and acid amides. The latter, though preferentially absorbed, were not absorbed by special cell activity. However, Höber and Höber, in their work with rat intestinal loops did not compare amino acids amongst themselves. Lathe (1943) concluded from his work with intestinal loops of dogs that the rate of absorption of amino acids decreases with

an increase in the molecular size. Kratzer (1944) found that the rate of absorption of amino acids from the gastrointestinal tract of the chick varied inversely with the apparent molal volume of the amino acids. From this he concluded that amino acid absorption in the chick is a function of the rate of diffusion of the amino acid and is not controlled by any cellular mechanism. Bolton and Wright (1937) analysed blood in the carotid artery, superior mesenteric vein, hepatic vein, inferior vena cava, and lymph in the cisterna chyli or thoracic duct in the cat. Absorption of amino acids from the intestine into the capillaries and lymphatics was found to be in accordance with the physical law of diffusion; there was no clear evidence to indicate any selective activity on the part of the capillary or lymphatic endothelium.

The small intestine is a striking example of a tissue which to some substances shows a selective activity, but which to other substances, even of the same chemical group, is quite inert. Two methods have been adopted to distinguish between the two groups of substances, namely (a) the effect of concentration of the solute on the rate of absorption, and (b) the effect of specific poisons on the rate of absorption.

The former method has been used by Höber and Höber (1937) to investigate absorption of amino acids from the

small intestine of the rat. They found that by varying the concentration of amino acids the percentage absorption from the small intestine was not constant but decreased with increasing concentration. Consequently they assumed that amino acids did not penetrate by simple diffusion but were preferentially absorbed by a cellular mechanism.

In the present work it has been found that in the anaesthetised cat the percentage absorption of isosmotic glycine from upper loops of the small intestine is 89.7% while the percentage absorption of half isosmotic glycine in the upper loops is 78.4%. Thus the percentage absorption increases slightly with increasing concentration. This is contrary to the findings of Höber and Höber (1937) in the rat. In the decerebrate cat the percentage absorption rates are equal for isosmotic and half isosmotic glycine from upper loops of the small intestine, being 78.3% and 77.8% respectively. This evidence seems to indicate that, unlike the rat, the cat does not absorb amino acids from the small intestine by a selective process involving a cellular mechanism. Kratzer (1944) also concluded that amino acids penetrated the small intestine of the chick by a process of diffusion since he found that the rate of absorption of amino acids was inversely proportional to their apparent molecular volume. Similarly in both anaesthetised and decerebrate

cats the rates of absorption were found to be inversely proportional to the apparent molal volumes of the amino acids. The milli molar absorption rates of the four amino acids studied in this work were found to be in the order, glycine, L(+) alanine, D(-)isoleucine, L(-)phenylalanine, i.e., as the apparent molal volume increases the rate of absorption decreases (Fig. 23). This also suggests that amino acids are not absorbed preferentially from the small intestine of the cat by a cellular mechanism.

In the rat under urethane anaesthesia it was found that the percentage absorption rate of isosmotic glycine was 65.2% in the upper loop of the small intestine and that the absorption rate of half isosmotic glycine was 68.3%. In the lower loop the percentage absorption rate of isosmotic glycine was 50% while the percentage rate of half isosmotic glycine in the lower loop was 66.4%. In the upper loop there seems to be no evidence for preferential absorption of glycine but in the lower loops the absorption rates decrease with increasing concentration suggesting preferential absorption takes place in this region of the gut. The evidence seems unsatisfactory. If the milli molar absorption rates of the four amino acids are examined it will be seen that in the upper loop they are in the order, glycine,

L(+) alanine, L(-) phenylalanine, D(-) isoleucine, and in the lower loop they are in the order, glycine, L(-) phenylalanine, L(+) alanine, D(-) isoleucine, i.e., the rates of absorption do not decrease with increasing molecular volume although there is a trend in that direction in the upper loop. This seems to confirm the findings of Höber and Höber (1937) who claim to furnish evidence for preferential absorption of amino acids in the rat.

On the other hand, it may be that a more extensive study of the relationship of absorption rates of amino acids and their apparent molal volumes from the small intestine of the rat would provide a different picture. When milli molar absorption rate is plotted against apparent molal volume (Fig. 17) the resulting graphs correspond in shape to the red dotted line in Fig.19 which represents the milli molar absorption rate of the amino acids from the small intestine of the chick plotted against apparent molal volume of these amino acids. Kratzer (1944), however, obtains a straight line when the milli molar absorption rates of a large number of amino acids are plotted against the apparent molal volume of these amino acids. Similarly, in the rat it might be

that a straight line would be obtained if the milli molar absorption rates of a large number of amino acids were plotted against the apparent molal volume of the amino acids. Such a straight line relationship indicates increasing rate of absorption with decreasing apparent molal volume, i.e., the amino acids would be absorbed by diffusion and not by a preferential mechanism. However, with the limited number of amino acids investigated at present it is impossible to draw this conclusion. In the graphs (Fig. 17) glycine, alanine, isoleucine, but not phenylalanine, conform to the trend of increasing rate of absorption with decreasing apparent molal volume. There is a characteristic upward gradient in the curve of each graph to the point corresponding to the absorption value of phenylalanine. Such an upward gradient occurs in Kratzer's graph (Fig.10) but despite this he concluded that absorption of amino acids in the chick is a process of diffusion.

In the cat, only in the graph for milli molar absorption rates in the upper loop of the decerebrate animal does this upward gradient appear for the point corresponding to the absorption value of phenylalanine. It can therefore be argued with more certainty that absorption of amino acids in the cat is a process of diffusion.

Mohl and Schmidt (1937) have recorded values of the diffusion coefficients of several amino acids at different temperatures. The diffusion coefficient is not a linear function of the temperature but increases with the temperature at an increasingly rapid rate as the temperature is increased. Temperature is known to play an important part in the functioning of the kidney. Bickford and Winton (1933) perfused an isolated kidney of a dog with defibrinated blood from a pump-lung circulation at a variable temperature and found that urine flow was increased by cold, while its composition came very closely to resemble that of a plasma-transudate, i.e., the chloride increased and the creatinine diminished. They explained this change as due to the depression of the metabolism of the tubule cells which became incapable of absorbing chloride and water.

If diffusion was the only factor involved in the absorption of amino acids from the small intestine one would therefore expect that the rate of absorption would be more rapid in animals having a higher body temperature e.g., birds whose body temperature in average health is 40°C (Starling, 1941). However this is not so in the case of absorption of amino acids.

Figures 24 and 25.

The general trend of the curves is horizontal. An increase of absorption with temperature would have been indicated by a sloping upwards of the curves to the right.

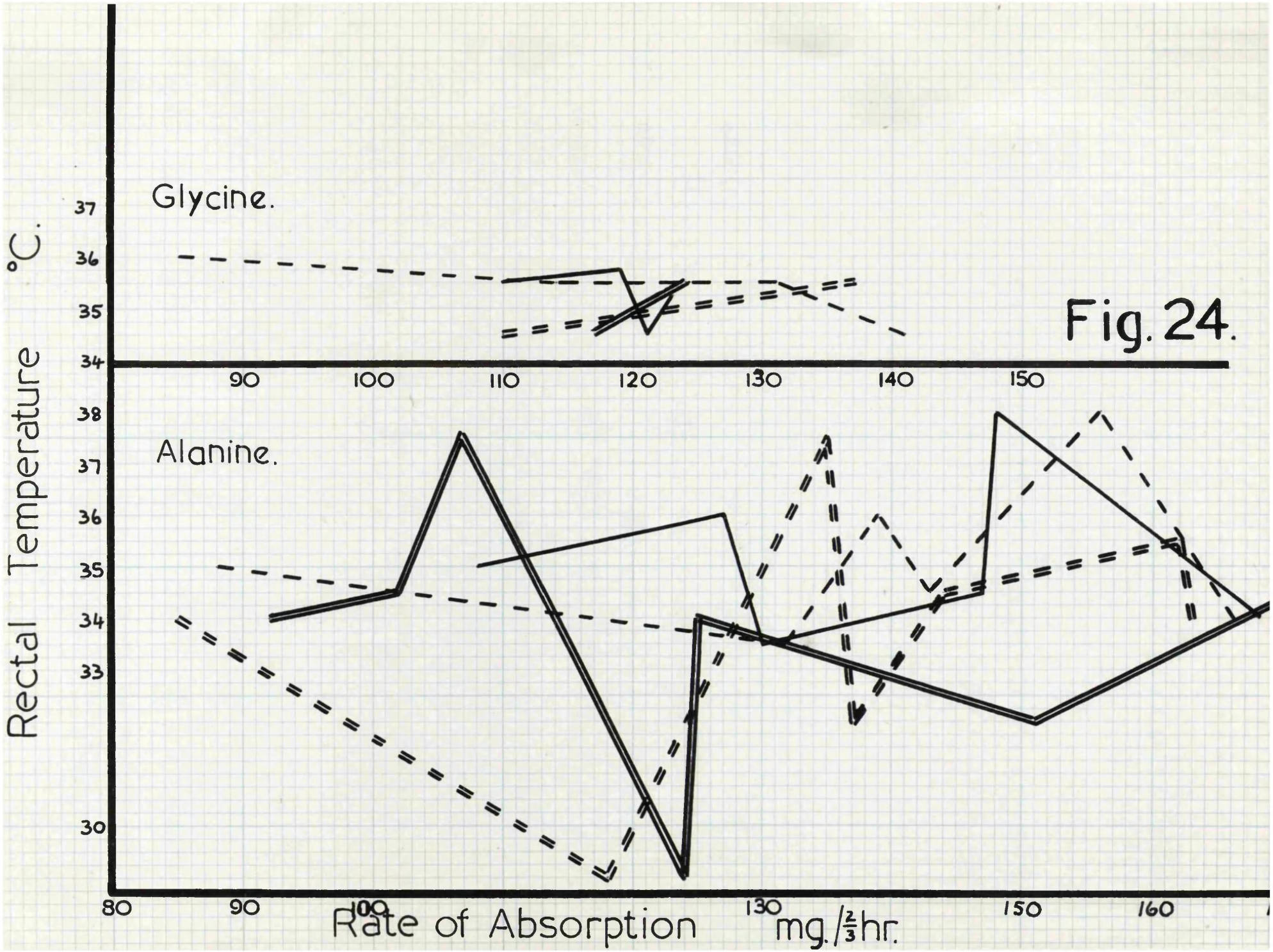

Glycine.
37
36
35
34
90
100
110
120
130
140
150
Alanine.
38
37
36
35
34
33
30
80
90
100
130
150
160
Rectal Temperature °C.
Rate of Absorption mg./⅔hr.

Fig. 24.

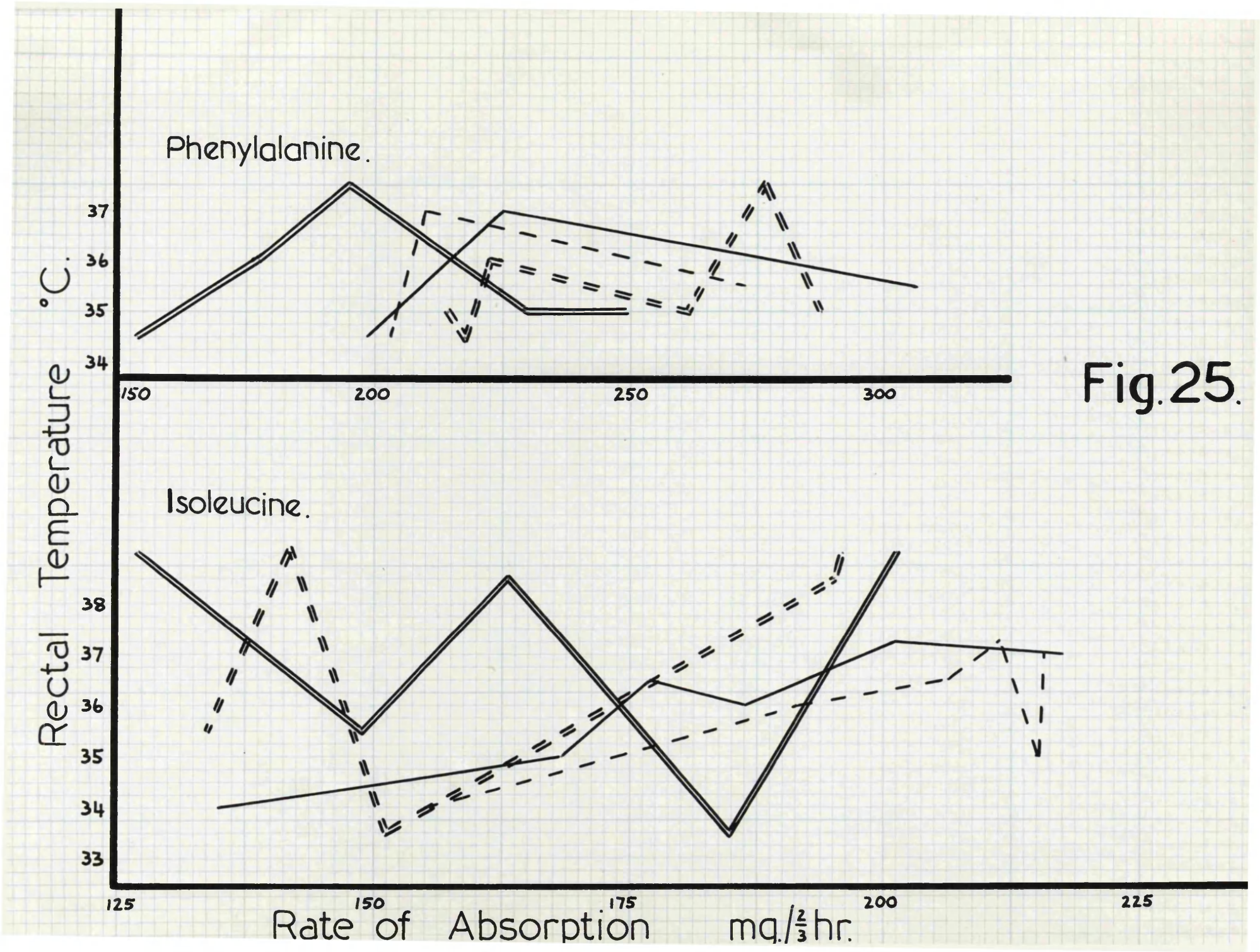

Phenylalanine.
Isoleucine.
Rectal Temperature °C.
37
36
35
34
38
37
36
35
34
33
150
200
250
300
125
150
175
200
225
Rate of Absorption mg./⅔ hr.

Fig. 25.

The absorption value for glycine in the gastrointestinal tract of the chick (Kratzer, 1944) is less than that reported for the rat (Wilson and Lewis, 1929; Cori, 1926-27). Similarly, variations in temperature in decerebrate cats in the present experiments did not bring about any corresponding changes in the rates of absorption of the amino acids from the small intestine. Admittedly these variations were, for the most part, very small (Figs. 24 and 25). However, Auchinachie, Macleod, and Magee (1930) found that the epithelium of isolated loops of the small intestine of rabbits allowed glucose to diffuse more rapidly "as its vitality is stimulated by a rise in temperature". They claim "that something in the nature of selective absorption comes into play as the temperature rises and that this property acts so as to accelerate diffusion....that when the selective factor comes into play it accelerates the rate of absorption for these substances, such as glucose, which are of physiological importance and probably retards that of those which are not required in the organism."

These generalisations do not apply to the absorption rates of amino acids whether it is claimed that amino acids are absorbed by diffusion or by selective action since the rate remains constant when the

temperature rises or falls within the range 29° - 40°C. It is essential in all such experiments where the temperature of the animal or the intestinal loop deviates markedly from the average to ensure that there is no subsequent desquamation of the epithelial cells. Obviously there is urgent need for research on the whole question of the effect of temperature on the rate of absorption of amino acids from the intestine to clear up the existing state of confusion.

It cannot be emphasised too strongly that generalisations about the mechanism of amino acid absorption from the intestine cannot be formulated from the results obtained from one particular species. It appears from the evidence given above that the amino acids are absorbed from the intestine of the cat under entirely different conditions from those in the rat. How amino acids are absorbed from the small intestine of man is as yet unknown.

The mechanism whereby this preferential absorption in the rat is brought about is still obscure. The fact that phlorhizin inhibits carbohydrate absorption and does not inhibit amino acid absorption (Wilson,1932) suggests that different mechanisms are involved.

Obviously molecular size and weight play little or no part as a large molecule, such as phenylalanine, is absorbed at a greater rate than isoleucine which has a smaller molecular volume.

Höber (1936a) concluded that amino acid absorption was too fast to be explained by diffusion. Mehl and Schmidt (1937), however, believe that the diffusion coefficient is not only a function of the volume of the molecule but of the shape as well and herein may lie the key to the whole problem. One can well imagine molecules of diverse molecular volume being absorbed through the striated border of the epithelium of the small intestine at equal rates because of some similarity in their spatial arrangement or configuration. There is little hope of any progress in this field until physical chemists can produce a relationship between the diffusion coefficient and the shape of the molecule. Such a relationship would be of incalculable value to the physiologist.

Recently Schofield and Lewis (1947) studied the relationship between stereochemical differences and rate of absorption of amino acids. They measured the rates of absorption of L-, D-, and Dl- alanine from the small intestine of the rat, using the Cori technique, and

found the L- and Dl- isomers were absorbed at the same rate while D-alanine was absorbed at a slower rate. The influence of the shift in position of the amino group ($-NH_2$) was also found by a comparison of α alanine and β alanine, and of Dl-serine and Dl-isoserine. As the amino group was removed from the carboxyl group, the rate of absorption was decreased. Similarly, the replacement of a hydrogen by a hydroxyl group, as shown by a comparison of α alanine and serine and of β alanine and isoserine, brought about a decreased rate of absorption.

Research along these lines could be profitably directed to a consideration of the absorption rates of the amino acids and their isomers for there is urgent need of a systematic investigation into the relationship between the rates of absorption of amino acids from the intestine and their molecular structure and configuration.

The final elucidation of the phenomena of absorption is still a long way off, but to attribute it to a vital process would be to admit defeat. The aim of physiologists is always to obtain a physico-chemical explanation of all the happenings in the body.

-----oOo-----

GENERAL SUMMARY.

GENERAL SUMMARY.

1. A brief historical introduction is given. It deals with the various theories concerning intestinal absorption, beginning about 460 B.C. with the speculations of Hippocrates.

2. Using ligatured loops in upper and lower regions of the small intestine in the anaesthetised rat, the absorption rate of isosmotic glycine was found.

3. Using the same technique the absorption rates of half isosmotic glycine, L(+) alanine, L(-)phenylalanine and D(-)isoleucine, from upper and lower loops of the small intestine of the anaesthetised rat were found. In the rat, half isosmotic glycine was absorbed from the small intestine at the same rate as isosmotic glycine.

4. In terms of unit mucosal surface area, isosmotic glycine and half isosmotic glycine, L(+)alanine, L(-) phenylalanine and D(-) isoleucine are more rapidly absorbed in the lower loops of the small intestine of the anaesthetised rat than in the upper loops. The relative rates of absorption were phenylalanine > isoleucine > glycine > alanine in both upper and lower loops.

5. In terms of milli molar rates of absorption, isosmotic glycine, half isosmotic glycine and L(+) alanine are absorbed more rapidly in the upper loops of the small intestine of the anaesthetised rat than in the lower loops whereas half isosmotic D(-) isoleucine and L(-)phenylalanine are absorbed more rapidly in the lower than in the upper loops. The relative rates of absorption were, in the upper loop, glycine > alanine > phenylalanine > isoleucine, and in the lower loop, glycine > phenylalanine > alanine > isoleucine.

6. Graphs were drawn for the absorption rates of half isosmotic glycine, L(+)alanine, L(-) phenylalanine and D(-)isoleucine from upper and lower loops of the small intestine of the anaesthetised rat plotting rate of absorption in mg. per hour against molecular weight and against apparent molal volume. The milli molar rate of absorption per hour was also plotted against molecular weight and apparent molal volume. The graphs were found to correspond in shape to that by Kratzer for the absorption rates of these amino acids from the small intestine of the chick.

7. Numerous attempts to decerebrate rats ended in failure.

8. Using ligatured loops in upper and lower regions

of the small intestine in the anaesthetised cat, the absorption rates of isosmotic glycine and its sodium salt were found; isosmotic glycine is absorbed at a much greater rate than its sodium salt.

9. Using the same technique, the absorption rates of half isosmotic glycine, L(+) alanine, L(-) phenylalanine and D(-) isoleucine from upper and lower loops of the small intestine of the anaesthetised cat were found. Isosmotic glycine is absorbed at a greater rate from the small intestine of the cat than half isosmotic glycine and thereby differs from the results in the rat. This suggests that amino acids may not be absorbed preferentially by cellular mechanism in the cat.

10. In terms of unit mucosal surface area isosmotic glycine and its sodium salt, and half isosmotic glycine, L(+) alanine, L(-) phenylalanine and D(-) isoleucine are absorbed more rapidly in the lower loops of the small intestine of the anaesthetised cat than in the upper loops. The relative rates of absorption were phenylalanine > isoleucine > alanine > glycine in both upper and lower loops.

11. In terms of milli molar rates of absorption, isosmotic glycine, half isosmotic L(+) alanine, L(-) phenylalanine and D(-) isoleucine are absorbed more

rapidly in upper loops of the small intestine of the anaesthetised cat than in the lower loops, whereas the isosmotic sodium salt of glycine and half isosmotic glycine are absorbed more rapidly in the lower than in the upper loops. The relative rates of absorption were glycine > alanine > isoleucine > phenylalanine in both upper and lower loops.

12. The small intestine of the cat absorbs glycine, alanine, phenylalanine and isoleucine at a faster rate than the small intestine of the rat even allowing for a greater degree of villous development in the small intestine of the cat.

13. Using ligatured loops in upper and lower regions of the small intestine in the decerebrate cat, the absorption rates of isosmotic glycine and its sodium salt were found; isosmotic glycine is absorbed at a much greater rate than its sodium salt.

14. Using the same technique, the absorption rates of half isosmotic glycine, L(+) alanine, L (-) phenylalanine and D (-) isoleucine from upper and lower loops of the small intestine of the decerebrate cat were found. Half isosmotic glycine is absorbed at a much slower rate from the small intestine of the decerebrate cat than isosmotic glycine.

15. In terms of mucosal surface area, isosmotic glycine and half isosmotic glycine, L(+) alanine, L (-) phenylalanine and D (-)isoleucine are absorbed more rapidly in the lower loops of the small intestine of the decerebrate cat than in the upper loops. The relative rates of absorption were, phenylalanine > isoleucine > alanine > glycine in both upper and lower loops.

16. In terms of milli molar rates of absorption, isosmotic glycine and half isosmotic L(-) phenylalanine and L(+)alanine are absorbed more rapidly in upper loops of the small intestine of the decerebrate cat than in the lower loops, whereas half isosmotic glycine is absorbed more rapidly in the lower than in the upper loops; the milli molar absorption rate of D(-) isoleucine is the same in both upper and lower loops. The relative rates of absorption were glycine > alanine > isoleucine > phenylalanine in both upper and lower loops.

17. Graphs were drawn for the absorption rates of half isosmotic glycine, L alanine, L - phenylalanine and D - isoleucine from upper and lower loops of the small intestine of (a) the anaesthetised cat and (b) the decerebrate cat, plotting the rate of absorption in

mg. per 40 minutes against molecular weight and apparent molal volume. The milli molar rate of absorption was also plotted against molecular weight and apparent molal volume. The graphs of (a) and (b) were found to be similar in shape.

18. Using ligatured loops in upper and lower regions of the small intestine in the anaesthetised cat, the absorption rates of a mixture of equal parts of half isosmotic glycine and half isosmotic glucose was found. Half isosmotic glycine is absorbed at a slightly faster rate than the glycine in the mixture.

19. A statistical analysis of all results was made.

20. Absorption is not depressed by the anaesthetic used.

21. It is safe to study absorption of amino acids from isosmotic and half isosmotic solutions.

22. The histological picture of each loop was examined to ensure that the epithelium of the small intestine was uninjured by the solute. Only in the case of the sodium salt of glycine was there extreme desquamation.

23. There are two schools of thought regarding the mode

of absorption of amino acids from the small intestine. Höber believes amino acids to be absorbed from the small intestine of the rat by preferential absorption involving a cellular mechanism. On the other hand, Kratser and Lathe believe amino acids are absorbed by a process of diffusion from the small intestine of the chick and the dog respectively.

24. An examination of these theories along with the observations reported in this thesis lends support to both schools of thought.

--------oOo--------

I wish to express my thanks to Professor R. C. Garry and to Professor G. H. Bell for their advice and constant encouragement.

"Nature! We are surrounded and embraced by her: powerless to separate ourselves from her, and powerless to penetrate beyond her ......

We live in her midst and know her not. She is incessantly speaking to us but betrays not her secret ......

She rejoices in illusion. Whoso destroys it in himself and others, him she punishes with the sternest tyranny. Whoso follows her in faith, him she takes as a child to her bosom.

She wraps a man in darkness and makes him forever long for light. She creates him dependent upon the earth, dull and heavy; and yet is always shaking him until he attempts to soar above it ......

She has brought me here and will also lead me away. I trust her. She may scold me, but she will not hate work. It was not I who spoke of her. No! What is false and what is true, she has spoken it all. The fault, the merit, is all hers ......

Everyone sees her in his own fashion. She hides

under a thousand names and phrases, and is always the same. "

- Goethe's Aphorisms, translated by Huxley.

-----oOo-----

B I B L I O G R A P H Y.

BIBLIOGRAPHY.

Abderhalden, E. Baumann, L. and London, E.S. (1907).
Hoppe - Seyl. Z. 51, 384. "Weitere Studien über die normale Verdauung der Eiweisskörper im Magendarmkanal des Hundes."

Abderhalden, E. Korosy, K. and London, E.S. (1907).
Hoppe - Seyl. Z. 53, 148. "Weitere Studien über die normale Verdauung der Eiweisskörper im Magendarmkanal des Hundes."

Abderhalden, E. Frym, O. and London, E.S. (1907).
Hoppe - Seyl. Z. 53, 326. "Über die Resorptionsverhältnisse von in den Magendarmkanal eingeführten Monoaminosäuren."

Abderhalden, E. London, E.S. and Voegtlin, C. (1907).
Hoppe - Seyl. Z. 53, 334. "Abbau des Diglycyl - glycins und der Biuretbase im Magendarmkanal des Hundes."

Abderhalden, E. and London, E. S. (1910).
Hoppe - Seyl. Z. 65, 251. "Weiterer Beitrag zur Frage nach dem Ab - und Aufbau der Proteine im

tierischen Organismus."

Abderhalden, E. (1912a).
Hopps - Seyl. Z. 78, 382. "Weiterer Beitrag zur Frage nach dem Schicksal der Eiweissabbau: :produkte im Darmkanal. Über das Vorkommen der einzelnen Amino-sauren in verschiedenen Teilen des Darmkanals."

Abderhalden, E. (1912b).
Synthese der Zellbausteine in Pflanze und Tier, Berlin: Springer.

Abderhalden, E. (1913).
Hoppe - Seyl. Z. 88, 478. "Der Nachweis von freien Aminosäuren im Blute unt normalen Verhältnissen".

Abel, J. J. (1913). Internat. Physiologen Kongress. cited by Verzar, F, and McDougall, E. J. in Absorption from the intestine. 1936. London: Longmans, Green & Co.

Abel, J. J. Rowntree, L.G. and Turner, B.B. (1913).
Trans. Assoc. Americ. Physicians. "On the removal of diffusibles from the circulating blood by means of dialysis."

Abel, J. J. Rowntree, L. G. and Turner, B.B. (1914).
J. Pharmacol. 5, 275. "On the removal of diffusible substances from the circulating blood of living animals by dialysis."

Althausen, T. L. (1939). Amer J. Dig. Dis. 6, 544.
"A test for intestinal absorption - Rats."

Andrews, J. C. and Johnston, C.G. (1933).
J. biol. Chem. 100, vii. "The absorption of Sulphur compounds from intestinal loops of dogs."

Andrews, J. C. and Johnston, C.G. (1933).
J. biol. Chem. 101, 635. "The absorption of Sulphur compounds from intestinal loops of dogs."

Andrews, J.C.Johnston, C.G. and Andrews, K.C. (1935).
J. biol. Chem. 109, iii. "The absorption and metabolism of cystine and of certain cystine derivatives in dogs with isolated intestinal loops."

Andrews, J. C. Johnston, C.G. and Andrews, K.C. (1936).
Amer. J. Physiol. 115, 188. "The absorption of cystine, methionine and cysteic acid from intestinal loops of dogs."

Auchinachie, D.W. Macleod, J.J.R. and Magee, H.E.(1930).
J. Physiol. 69, 185. "Studies on diffusion through surviving isolated intestine."

Beaumont. (1833). Cited by Slyke, D.D.Van. in Arch. intern. Med. (1917), 19, 56.

Bell, D. J. Horne, E.A. and Magee, H.E. (1933).
J. Physiol. 78, 196. "Part I. Decerebration of the rat under local anaesthesia and the use of the preparation for absorption studies."

Berg, C.L.R. and Baughers, L.C. (1932).
J. biol. Chem. 98, 171. "Tryptophan metabolism: The rate of absorption of l and dl Tryptophan and Tryptophan derivatives from the gastro intestinal tract of the rat".

Bickford, R.G. and Winton, F.R. (1933).
J. Physiol. 78, 14P. "The influence of temperature on the isolated mammalian kidney",

Bjerrum, N. (1923).
Z. phys. Chem. 104, 147. "Die Konstitution der Ampholyte, besonders der Aminosäuren, und ihre Dissociation-skonstanten."

Bolton, C. and Wright, C.P. (1937).
J. Physiol. 89, 269. "The absorption of amino acids and their distribution in the body fluids."

Braus, H. (1924).
Anatomie des Menschen. Berlin: Springer.

Brettauer, J. and Steinach, S. (1887).
Moleschotts Untersuchungen. 3, 173. "Untersuchungen über das Cylinderepithelium der Darmzotten und seine Beziehung zur Fettre-sorption."

Brücke, E. (1851).
S.B.Akad. Wiss. Wien. 6, 214. "Über ein in der Darmschleimhaut aufgefundenes Muskelsystem."

Brücke, E. (1852).
S.B.Akad, Wiss, Wien. 9,900. "Über die Aufsaugung des Chylus aus dem Darmhöhle."

Brücke, E. (1854).
Denkschr. Akad. Wiss, Wien. 6, 99. "Über die Chylusgefässe und die Resorption des Chylus."

Cathcart, E.P. and Leathes, J.B. (1906).
J. Physiol. 33, 402. "On the absorption of

proteins from the intestine."

Cathcart, E.P. (1912).

The physiology of protein metabolism. London: Longmans, Green & Co.

Chambers, E. G. (1946).

Statistical calculation for beginners. Cambridge University Press.

Chase, B.W. (1933).

J. biol. Chem. 100, xxvii. "The absorption of leucine, valine and their isomers from the gastrointestinal tract of the white rat."

Chase, B.W. and Lewis, H.B. (1933).

J. biol. Chem. 101, 735. "The metabolism of Sulphur: Rate of absorption of dl-Methionine from the gastrointestinal tract of the white rat."

Chase, B.W. and Lewis, H.B. (1934).

J. biol. Chem. 106, 315. "The rate of absorption of leucine, valine, and their isomers from the gastrointestinal tract of rats."

Cohn, E.J. McMeekin, T.L. Edsall, J.T. and Blanchard, M.H.

(1934).

J. Amer. chem. Soc. 56, 784. "Studies in the physical chemistry of amino acids peptides and related substances: The apparent molal volume and the electrostriction of the solvent."

Cohn, E.J. et al. (1936).

J. Amer. chem. Soc. 58, 415. "Studies in the physical chemistry of amino acids, peptides, and related substances: The densities and viscosities of aqueous solutions of amino acids."

Cohn, E.J. and Edsall, J.T. (1943).

Proteins, Amino acids, and peptides as ions and dipolar ions. New York : Reinhold Publ. Corpn.

Cohnheim, O. (1898).

Z. Biol. 36, 128. "Ueber Dünndarmresorption."

Cohnheim, O. (1899).

Z. Biol. 37, 443. "Ueber die Resorption im Dünndarm und der Bauchhöhle."

Cohnheim, O. (1899).

Z. Biol. 38, 419. "Versuche am isolirten überlebenden Dünndarm."

Cohnheim, O. (1900).

Z. Biol, 39 , 187. "Ueber Dünndarmresorption."

Cohnheim, O. (1901).

Hoppe - Seyl. Z. 33, 451. "Die Umwandlung des Eiweiss durch die Darmwand."

Cohnheim, O. (1912).

Hoppe - Seyl. Z. 76, 293. "Zur Frage der Eiweissresorption."

Cohnheim, O. (1913).

Hoppe - Seyl. Z. 84, 419. "Die Wirkung vollständig abgebauter Nahrung auf den Verdauungskanal."

Collander, R. and Berlünde, H. (1933).

Acta. Bot. Fenn. 11 cited by Höber, R. and Höber, J. (1937) in J. cell. comp. Physiol, 10, 401

Cori, C. F. (1925).

J. biol. Chem. 66, 691. "The fate of sugar in the animal body."

Cori, C.F. (1926-27).

Proc. Soc. exp. Biol., N.Y. 24, 126. "The absorption of glycine and dl- alanine".

Czerny, V. and Latschenberger, J. (1874).
Virchows Arch. 59, 161. "Physiologische Untersuchungen über die Verdauung und Resorption im Dickdarm des Menschen".

Dalton, J.B. and Schmidt, C.L.A. (1933).
J. biol. Chem. 103, 549. "The solubilities of certain amino acids in water, the densities of their solutions at 25°C and the calculated heats of solution and partial molal volumes".

Dalton, J.B. and Schmidt, C.L.A. (1935).
J. biol. Chem. 109, 241. "The solubilities of certain amino acids in water, the densities of their solutions at 25°C and the calculated heats of solution and partial molal volumes".

Davidson, J.N. and Garry, R.C. (1940).
J. Physiol. 97, 509. "The absorption of monosaccharides from the distal small intestine of anaesthetised cats."

Delaunay, H. (1910).
Thèse de Bordeaux. cited by Verzar, F and McDougall, E.J. in Absorption from the intestine. 1936. London: Longmans, Green and Co.

Delaunay, H. (1913a).

C.R.Soc. Biol, Paris. 74, 641. "Sur l'azote restant du plasma de quelques vertébrés".

Delaunay, H. (1913b)

C. R. Soc. Biol., Paris. 74, 764. "Sur l'azote restant du sang avant et pendant l'absorption intestinale de l'azote alimentaire".

Delaunay, H. (1913c).

C.R. Soc. Biol., Paris. 74, 769. "Sur l'azote restant du sang avant et pendant l'absorption d'un melange d'acides aminés introduit dans l'intestin."

Doty, J. R. and Eaton, A.G. (1937a).

J. biol. Chem. 122, 139. "Metabolism of the basic amino acids : Rates of absorption in rats of the mono hydrochlorides of d-lysine and l-Histidine".

Doty, J.R. and Eaton, A.G. (1937b).

Amer. J. Physiol, 119, 296. "The metabolism of lysine: Studies on the heat production and blood and urine constituents after administration of lysine monohydrochloride to the dog."

Doty, J.R. and Eaton, A.P. (1933).
Amer. J. Physiol. 105, 53. "Rate of absorption of the basic amino compounds as a function of the amount Fed."

Dutrochet, M.H. (1826).
Agent immediat du mouvement vital. Paris: Dentu.

Espé, D.L. and Cannon, C.Y. (1932).
Anat. Rec. 53, 367. "The premortem length of the intestine of a calf."

Espé, D.L. and Cannon, C.Y. (1940).
J. Dairy Sc. 23, 1211. "The length of the intestine of calves and its bearing on the absorption of the nutrients from the chyme."

Fick, A. (1855).
Ann. Chim. (Phys). 94, 59. cited by Mohl and Schmidt (1937) in Univ. Calif. Publ. 8 165.

Fischer, H.W. (1922).
Ann. Chim. (Phys) 72, 339. "Ueber die Wiederherstellung eines Metalls durch einanderes und über die Eigenschaft der thierischen Blase

Flussigkeiten durch sich hindurch zu lassen, und sie in einigen Fällen anzuheben."

Fisher, R.A. and Yates, F. (1943).
Statistical tables for biological agricultural and medical research. 2nd. ed. p. 30. London: Oliver and Boyd, Ltd.

Fleischmann, W. (1928).
Pflug. Arch. ges Physiol. 220, 448. cited by Höber and Höber, J. (1937) in J. cell comp. Physiol. 10, 401.

Folin, O. and Denis, W. (1912a).
J. biol. Chem. 11, 87. "Protein metabolism from the stand point of blood and tissue analysis."

Folin, O. and Denis, W. (1912b).
J. biol. Chem. 11, 161. "Protein metabolism from the stand point of blood and tissue analysis: Origin and significance of the ammonia in the portal blood".

Folin, O. and Denis, W. (1912c).
J. biol. Chem. 12, 141. "Protein metabolism from

the stand point of blood and tissue analysis: Further absorption experiments with especial reference to the behaviour of creatine and creatinine and to the formation of urea."

Folin, O. and Denis, W. (1912d).
J. biol. Chem. 11, 503. "An apparatus for the absorption of fumes."

Folin, O. and Denis, W. (1912e).
J. biol. Chem. 11, 527. "New methods for the determination of total nonprotein nitrogen, urea and ammonia in blood".

Folin, O. and Farmer, C.J. (1912f).
J. biol. Chem. 11, 493. "A new method for the determination of total nitrogen in urine."

Folin, O. (1912g).
J. biol. Chem. 11, 507. "On the determination of urea in urine."

Folin, O. and Macallum, A.B. (1912h).
J. biol. Chem. 11, 523. "On the determination of ammonia in urine."

Folin, O. and Marenzi, A.D. (1929).
J. biol. Chem. 83, 103. "An improved colorimetric method for the determination of cystine in Proteins."

Frazer, A.C. and Stewart, H.C. (1938).
J. Physiol, 94, 24P. "Evidence of fat/fatty acid partition in human fat absorption."

Frazer, A.C. (1940).
Physiol. Rev. 20, 561. "Fat absorption and its relationship to fat metabolism."

Frazer, A.C. Stewart, H.C. and Schulman, J.H. (1942).
Nature, Lon. 149, 167. "Emulsification and absorption of fats and paraffins in the intestine."

Friedlander, G. (1896).
Z. Biol. 33 , 64. "Ueber die Resorption gelöster Eiweisstoffe im Dünndarm."

Furth, O. and Friedmann, M. (1908).
Arch. exp. Path. Pharmak. Suppl. p. 214.
"Über die Resorptions-weise jodierte Eiweisskorper."

Galen. (A.D.130 - 200). cited by Longet, F.A. in
Traité de Physiologie, (1868). 3rd. ed. 1, 580.

Gallo, G. and Alieri, V. (1938).
Boll. Soc. ital. Biol. sper. 13, 188.
"Velocita di assorbimento di soluzione di peptone e di amino acidi."

Garry, R.C. and Smith, L.A. (1943).
J. Physiol, 101, 484. "Factors affecting absorption of sodium dl-mandelate from the intestine of cats."

Gayda, T. (1915).
Arch. Fisiol. 13, 83. "Contributo allo studio dell' assorbimento intestinale dei prodotti di idrolisi delle sostanze proteiche. Ricerche sull'intestino tenue sopravvivente perfuso con liquido di Tyrode."

Greene, E.C. (1935).
The anatomy of the rat. Fig. 113. Philadelphia: Trans. Amer. Phil. Soc.

Gumilewski, S. (1886).
Pflüg. Arch. ges. Physiol, 39, 556. "Ueber Resorption im Dünndarm."

Hamburger, H.J. (1896).
Arch. Anat. Physiol. Lpz. 36, 248. "Ueber den

Einfluss des intraintestinalen Drucks auf die Resorption im Dünndarm."

Hamburger, H. C. (1896).

Zbl. Physiol, 9, 647. "Ueber bei Bedeutung von Athmung und Peristaltik für die Resorption im Dünndarm."

Hamburger, H. J. (1899).

Arch. Anat. Physiol., Lpz. 431 suppl. "Ueber den Einfluss von Salzlösungen auf das Volum thierischer Zellen."

Hamburger, H.J. (1904).

Osmotischer Druck und Ionenlehre 2, 167. "Resorption in mit Epithel ausgekleideten Höhlen: Resorption im Darm."

Hamburger, H.J. (1904).

Osmotischer Druck and Ionenlehre, 3, 7. "Osmotischer Verhalten verschiedener isolirter Zellen 2. Epithel. Die Volumveränderunger isolirter Epithelzellen und deren Kerne unter dem Einfluss anistonischer Salzlosungen."

Heidenhain, R. (1888).

Pflüg. Arch. ges. Physiol, 43, 63. "Beitrage zur

Histologie und Physiologie der Dünndarmschleimhaut."

Heidenhain, R. (1894).

Pflüg. Arch. ges. Physiol, 56, 579. "Neue Versuche über die Aufsaugung im Dünndarm."

Heller, H. and Smirk, F.H. (1932).

J. Physiol. 76, 283. part IV. "Studies concerning the alimentary absorption of water and tissue hydration in relation to diuresis: the influence of anaesthetics and hypnotics on the absorption and excretion of water."

Henle, J. (1837).

Symbolae ad Anat. Villorum. Berlin: Halle.

Hiller, A. and Slyke, D.D.Van. (1922).

J. biol. Chem. 53, 253. "A Study of certain protein precipitants."

Hippocrates (BC 460 - 377 or 359). cited by Longet, F.A. in Traité de physiologie (1868). 3rd. ed. 1,380.

Höber, R. (1898).

Pflüg Arch. ges. Physiol. 70, 624. "Ueber Resorption im Dünndarm".

Höber, R. (1899).

Pflüg. Arch. ges Physiol. 74, 245. "Ueber Resorption im Dünndarm."

Höber, R. (1901).

Pflüg. Arch. ges. Physiol. 86, 199. "Ueber Resorption im Darm."

Höber, R. (1903).

Pflüg. Arch. ges, Physiol, 94, 337. "Ueber Resorption im Darm."

Höber, R. (1906).

Biol. Zbl. No. 21 abstr. Zbl. Biochem. Biophys. (1907). 6, 227. "Zur Frage der electwen Fähigkeiten der Resorptionsorgane."

Höber, R. (1914).

Physikalische Chemie der Zelle und der Gewebe. Berlin.

Höber, R. and Orskov, S.L. (1933).

Pflüg. Arch. ges. Physiol. 231, 599. "Untersuchungen über die Formeiergeschwindigkut von Anolekroly:ten bei roten Blutkörperchen verschiedener Tiererarten."

Höber, R. (1936a).

Amer. J. Physiol. 116, 78. "On the nature of intestinal absorption of amino acids."

Höber, R. and Höber, J. (1936a).

Proc. Soc. exp. Biol., N.Y. 34, 486. "Intestinal absorption of amino acids."

Höber, R. (1936b).

Physiol. Rev. 16, 52. "Membrane permeability to solutes in its relations to cellular physiology."

Höber, R. and Höber, J. (1936b).

Amer. J. med. Sc. 191, 873. "On the nature of intestinal absorption of amino acids."

Höber, R. and Höber, J. (1937).

J. cell. comp. Physiol, 10, 401. "Experiments on absorption of organic solutes in the small intestine of rats."

Hofmeister, F. (1882).

Hoppe - Seyl, Z. 6, 69. "Zur Lehre vom Pepton. Das Verhalten des Peptons in der Magenschleimhaut."

Hoppe - Seyler, F. (1881).

Hoppe - Seyl. Z. part 2, 348. "Ueber den Harnstoff in der Leber."

Hufner, G. (1897).

Ann. Chim. (Phys). 296, 134. cited by Mehl, J.W. and Schmidt, C.L.A. in Univ. Calif. Publ.(1937). 8, 181.

Internat. Crit. Tables (1929).

3, 111. New York: McGraw Hill, cited by Mehl, J.W. and Schmidt, C.L.A. in Univ. Calif. Publ. (1937) 8, 182.

Johnston, M.W. and Lewis, H.B. (1928).

J. biol. Chem. 78, 67. "Comparative studies in the metabolism of amino acids: Changes in the non protein nitrogenous constituents of the blood following administration of amino acids."

Kolliker, A. (1856).

Verh. Physik-Med. Wurzburg. 6 and 7 cited by Reid E.W. (1900) in Philos. Trans. B 192, 211

Kopac, M.J. and Chambers, R. (1937).

J. Cell. comp. Physiol. 9, 331. "The coalescence

of living cells with oil drops."

Kopac, M.J. (1937).
Biol. Bull. Woods Hole 73, 363. "The coalescence of a plant cell with oil drops."

Kopac, M. J. (1940).
Cold Spr. Harb. Sym. quant. Biol 8, 154.
"The physical properties of the extraneous coats of living cells."

Körösy, K. (1911).
Mag. orrosi. Arch. 12, 101, cited by Zbl. Biochem. Biophys. 12. No. 231. "Neue Beiträge und das Schicksal der parenteral eingefuhrten Eiweisskörper."

Kostyal, L. (1926).
Magy. orv. Arch. 27, 276. "A bélcsatorna vegyhátásanak jelentösége a zsirok felsivódására."

Kratzer, F.H. (1944).
J. biol. Chem. 153, 237. "Amino acid absorption and utilisation in the chick."

Kühne, W. (1867).
Virchows Arch. 39, 130. "Ueber die Verdauung

der Eiweisstoffe durch den Pankreassaft."

Kuthy, A. (1930).

Pflüg. Arch. ges Physiol. 225, 567. "Resorption, BlutNH_2-N und spezifisch dynamische Wirkung nach Fleischnahrung."

Kutscher, F. and Seemann, J. (1901).

Hoppe - Seyl. Z. 34, 527. "Zur Kenntnis der Verdauungsvorgänge im Dünndarm."

Kutscher, F. and Seeman, J. (1906).

Hoppe - Seyl. Z. 49, 298. "Beitrage zur Kenntnis der Verdauung in Dünndarm."

Landis, E.M. (1930).

Amer. J. Physiol. 93, 353. "The capillary blood pressure in mammalian mesentery as determined by the micro-injection method."

Laszt, L. (1938).

Pflüg Arch. ges. Physiol. 240, 636 "Die Resorption der Aminosäuren aus dem Darm nach Nebenniorenestirpation."

Lathe, G.H. (1943).

Rev. Canad. Biol. 2, 134. "The effect of concentration of different substances in intestinal contents on their absorption from the small intestine."

Lawrie, N.R. (1932).

Biochem. J. 26, 435. "The metabolism of dl-cystine in the rat."

Leubuscher, G. (1885).

Jenaische Ztschr. 18, 808. "Studien über Resorption seitens des Darmkanals."

Levene, P.A. and Kober, P.A. (1908-09).

Amer. J. Physiol. 23, 324. "The elimination of total nitrogen, urea, and ammonia following the administration of glycocol, asparagine and glycyl-glycin-anhydride."

Levene, P.A. and Meyer, G.M. (1909-10).

Amer. J. Physiol. 25, 214. "The elimination of total nitrogen, urea and ammonia following the administration of some amino acids, glycylglycin, and glycylglycin-anhydride."

Lieberkühn, J.N. (1745).

"De fabrica et actione villorum intestinorum tenuium". Leyden. cited by Franklin, K.J. (1933) in A short history of physiology. London: Bale, Sons and Danielsson.

Liebig, J. (1856).

Complete works on Chemistry. cited by Underhill, F.P. in The physiology of the amino acids (1915). Yale University Press.

London, E.S. and Kotschneff, N.P. (1934).

Hoppe - Seyl. Z. 228, 235. "In welcher Form wird Nahrungseiweiss resorbiert."

Longet, F.A. (1868).

Traité de Physiologie, Paris. 3rd. ed. 1, 380.

Ludwig, C. (1851).

Z. Rat. Med., Heidelberg 1, 255. "Neue Versuche uber die Beilhilfe der Nerven zur speichelabsonderung."

Macklin, C.E. and Macklin, M.T. (1926).

J. Anat., Lond - 61, 144. "Is the Mingazzini phenomenon in the villus of the small intestine an evidence of absorption?"

Magendie, F. (1825).

Précis Élémentaire de Physiologie, Paris. 2, 180. cited by Reid, E.W. (1900) in Philos. Trans. B 192, 211.

Markham, R. (1942).

Biochem. J. 36, 790. "A steam distillation apparatus suitable for micro Kjeldahl analysis."

McDougall, E.J. and Verzar, F. (1935).

Pflüg. Arch. ges. Physiol. 236, 321. "Die Resorption von wasser aus Kochsalz und Zuckerlösungen."

Mehl, J.W. and Schmidt, C.L.A. (1937).

Univ. Calif. Publ. 8, 165. "The diffusion of certain amino acids in aqueous solution."

Mering, J. (1907).

Lehrbuch der inneren Medizin. 4 Aufl. Jena:Fischer.

Moore, B. (1921).

Biochemistry p. 318. London: Arnold. "A study of the origin, reactions and equilibria of living matter".

Mottram, J.C. Cramer, W. Drew, A.H. (1922).

Brit. J. exp. Pathl 3, 179. "Vitamins,

exposure to Radium and intestinal fat absorption."

Müller, J. (1841).

Handbuch der Physiologie. Berlin.

Munk, J. (1897).

Lehrbuch der physiol. Chem. 4 Aufl. p. 199.

Nagano, J. (1902).

Pflüg. Arch. ges. Physiol 90, 389. "Zur Kenntnis der Resorption einfacher, im besonderen stereoisomerer Zucker im Organismus."

Neumeister, R. (1897).

Lehrbuch der physiol. Chem. 2 Aufl. p.299. cited by Verzar, F.and McDougall, E.J. in Absorptionfrom the intestine (1936). London: Longmans, Green and Co.

Öholm, L.W. (1910).

Z. phys. Chem. 70, 401. "Die freie Diffusion der Nichtelektrolyte. Über die Hydrodiffusion einiger optisch aktiven Substanzen."

Okuda, Y. (1925).

J. Biochem., Tokyo. 5, 217. " A new method for the determination of cystine in proteins (the iodine method).

Overton, E. (1897).

Z. phys. Chem. 22, 189. "Ueber die osmotischen Eigenschaften der Zelle in ihrer Bedeutung für die Toxikologie und Pharmakologie mit besonderer Berücksichtigung der Ammoniake und Alkaloide."

Overton, E. (1899).

Vierteljahrsschr. Ges. Zurich Jahrg. 44, 88. "Ueber die allgemeinen osmotischen Eigenschaften der Zelle, ihre vermutlichen Ursachen und ihre Bedentung für die Physiologie."

Pfluger, E. (1893) cited by Cathcart, E.P. (1912) in "The physiology of protein metabolism." London: Longmans, Green and Co.

Pflüger, E. (1901).

Pflüg. Arch. ges. Physiol. 86, 1. "Die Resorption der Fette vollzieht sich dadurch, dass sie in wässrige Lösung gebracht werden."

Rappaport, F. and Eichhorn, F. (1947a).

J. Lab. clin. Med. 32, 1034. "Rapid titrimetric method for the determination of non protein nitrogen."

Rappaport, F. and Eichorn, F. (1947b).
Lancet No. V of 11. "Micro estimation of non protein nitrogen in serum, plasma, or blood in 5 - 10 minutes."

Reid, E.W. (1890).
J. Physiol. 11, 312. "Osmosis experiments with living and dead membranes."

Reid, E.W. (1892).
Brit. Med. J. 1, 1133. "Preliminary report on experiments upon intestinal absorptionwithout osmosis."

Reid, E.W. (1896).
J. Physiol. 20, 298. "The influence of the mesenteric nerves on intestinal absorption."

Reid, E.W. (1897).
J. Physiol. 21, 408. "A comparison of the diffusion into serum and absorption by the intestine of peptone and glucose."

Reid, E.W. (1898a).
Brit. Med. J. 2, 776. "Introduction to a discussion on intestinal absorption."

Reid, E.W. (1898b).
J. Physiol. 22, lvi. "Intestinal epithelium and absorption."

Reid, E.W. (1900).
Philos. Trans. B.192, 211. "On intestinal absorption, especially on the absorption of serum, peptone and glucose."

Reid, E.W. (1901a).
J. Physiol. 26, 427. "Intestinal absorption of maltose."

Reid, E.W. (1901b).
J. Physiol, 26, 436. "Transport of fluid by certain epithelia."

Reid, E.W. (1902).
J. Physiol. 28, 241. "Intestinal absorption of solutions."

Remmert, L.F. and Butts, J.S. (1942).
J. biol. Chem. 144, 41. "Amino acid metabolism in the rat."

Robinson, C.S. (1935).
J. biol. Chem. 108, 495. "The hydrogen ion

concentration of the contents of the small intestine."

Röhmann, F. (1887).

Pflüg. Arch. ges. Physiol. 41, 411. "Ueber Secretion and Resorption im Dünndarm."

Rudolphi, K.A. (1800).

Reil's Arch. 4, 63. "Einige Beobachtungen über die Darmzotten."

Salkowski, E. and Leube W. (1880).

Die Lehre vom Harn. p. 430. Berlin: Hirschwald.

Salvioli, G. (1880).

Arch. Anat. Physiol., Lpz. Suppl. p. 95. "Eine neue Methode für die Untersuchung der Functionen des Dünnderms."

Sarzana, G. (1933).

Boll. Soc, ital. Biol. sper. 8, 1235. "Sull' assorbimento delle sostanze azotate: assorbimento di soluzioni di aminoacidi introdotte in un'ansa alla vella."

Schmengler, F.E. (1933).

Pflüg. Arch. ges. Physiol. 232, 591. "Über die

Permeabilität getrocknetor Kollodiummembranen für Amino-säuren im Vergleich zu organischen Nichtelektrolyten."

Schmidt, C.F. (1923).

J. exp. Med. 37, 43. "A new method for mammalian decerebration."

Schmidt - Mulheim, A (1897).

Virchows Arch. 39. "Untersuchung über die Verdauung der Eiweisskorper."

Schofield, F.A. and Lewis, H.B. (1947).

J. biol. Chem. 168, 439. "A comparitive study of the metabolism of α alanine, β alanine, serine and isoserine: absorption from the gastrointestinal tract."

Seth, T.N. and Luck, J.M. (1925).

Biochem. J. 19, 366. "The relation between the metabolism and the specific dynamic action of amino acids."

Sharpey - Schafer, E. (1934).

The essentials of Histology 13th ed. pp. 369-380. London: Longmans, Green and Co.

Sheridan-Lea, A. (1890).

J. Physiol. 11, 226. "A comparitive study of artificial and natural digestions."

Shore, L.E. (1890).

J. Physiol. 11, 528. "On the fate of peptone in the lymphatic system."

Slyke, D.D.Van (1911).

J. biol. chem. 9, 185. "A method for quantitative determination of aliphatic amino groups. Applications to the study of proteolysis and proteolytic products."

Slyke, D.D. Van (1912).

J. biol. Chem. 12, 275. "The quantitative determination of aliphatic amino groups."

Slyke, D.D. Van and Meyer, G.M. (1912).

J. biol. Chem. 12, 399. "Amino nitrogen of the blood."

Slyke, D.D. Van (1913-14).

J. Biol. Chem. 16, 121. "The gasometric determination of aliphatic amino nitrogen in minute quantities."

Slyke, D.D.Van(1913-14).

J. biol. Chem. 16, 125. "Improved methods in the

gasometric determination of free and conjugated amino acid nitrogen in the urine."

Slyke, D.D.Van (1915).
J. biol. Chem. 23, 407. "A note on the micromethod for gasometric determination of aliphatic amino nitrogen."

Slyke, D.D. Van (1917).
Arch. intern. Med. 19, 56. "The present significance of the amino acids in Physiology and Pathology."

Slyke, D.D. Van (1929).
J. biol. Chem. 83, 425. "Manometric determination or primary amino nitrogen and its application to blood analysis."

Spallanzani (1780) cited by Slyke, D.D. Van (1917) in Arch. intern. Med. 19, 56.

Starling, E.H. (1896).
J. Physiol. 19, 312. " On the absorption of fluids from connective tissue spaces."

Starling, E.H. (1941).
Principles of Human Physiology (Lovatt Evans).
7th Ed. p. 972. London: Churchill.

Stearns, G. and Lewis, H.B. (1930).
J. biol. Chem. 86, 93. "The metabolism of Sulphur: the rate of oxidation of ingested cystine in the organism of the rabbit."

Sullivan, M.X. (1929a).
Pub. Health Rep. 44, 1421. (No. 24).
"Studies on the biochemistry of Sulphur: further studies on the distinctive reaction for cysteine and cystine."

Sullivan, M.X. and Hess, W.C. (1929b).
Pub. Health. Rep. 44, 1599. (No. 27). "Studies on the biochemistry of Sulphur: chemical groups involved in the naphthoquinone reaction for cysteine and cystine."

Sullivan, M.X. and Hess, W.C. (1931).
Pub. Health Rep. Suppl. U.S.P.H.S. 89. "The rate of absorption of cystine from the gastro: :intestinal tract of the white rat."

Thomson, W. (1936).

J. Hyg., Camb. 36, 24. "Stock diets for rats."

Tiedemann, and Gmelin (1820).

Versuche über die Wege auf Welchen Substanzen aus dem Darmkanal in das Blut gelangen. Heidelberg. cited by Reid, E.W. (1900) in Philos. Trans. B.192, 211.

Traube, J. (1899).

Samml. chem.u.chem-techn. 4, 275. "Ueber den Raum der Atome."

Verzár, F. and Kuthy, A. (1929a).

Biochem Z. 205, 369. "Die Bedeutung der Gallensäuren für die Fettresorption."

Verzár, F. and Kuthy, A. (1929b).

Biochem, Z. 210, 265. "Die Verbindung der gepaarten Gallensäuren mit Fettsäuren und ihre Bedeutung für die Fettresorption."

Verzár, F. and Kuthy, A. (1929c).

Biochem, Z. 210, 281. "Die Verbindung der gepaarten Gallensäuren mit Fettsäuren und ihre Bedeutung für die Fettresorption."

Verzar, F. and Kuthy, A. (1931).
Biochem. Z. 230, 431. "Die Bedeutung der gepaarten Gallensäuren für die Fettresorption."

Verzár, F. (1935).
Biochem. Z. 276, 17. "Die Rolle von Diffusion u. Schleimhautaktivität bei der Resorption von verschiedenen Zuckern aus dem Darm."

Verzár, F. and McDougall, E.J. (1936).
Absorption from the intestine. London: Longmans, Green and Co.

Verzár, F. and Wirz, H. (1937).
Biochem. Z. 292, 174. "Weitere Untersuchungen über die Bedingungen der selektiven Glucoseresorption."

Voit, C. (1867). cited by Cathcart, E.P. (1912) in The physiology of protein metabolism. London: Longmans, Green and Co.

Voit, C. and Bauer, J. (1869).
Z. Biol. 5, 363. "Ueber die Aufsaugung im Dick- und Dünndarm."

Walker, J. (1904a).

Proc. Roy. Soc. A 73, 155. "Theory of amphoteric electrolytes."

Walker, J. (1904b).

Proc. Roy. Soc. A 74, 271. "Theory of amphoteric electrolytes."

Wallace, G. B. and Cushny, A.R. (1898).

Amer. J. Physiol. 1, 411. "On intestinal absorption and the saline cathartics."

Wallace, G.B. and Cushny, A.R. (1899).

Pflüg. Arch. ges. Physiol. 77, 202. "Ueber Darmresorption und die salinischen Abführmittel."

Watzadse, G. (1929).

Pflug. Arch. ges. Physiol. 222, 640. "Physikalisch-chemische Untersuchungen zur Frage der Stoffverteilung zwischen Zelle und Umgebung."

Westenbrink, H.G.K.(1934).

Arch. néerl. Physiol. 19, 563. "Über Die Anpassung Der Darmresorption an Die Zusammensetzung Der Nahrung."

Wilson, R.H. and Lewis, H.V. (1929).
J. biol. Chem. 84, 511. "Rate of absorption of amino acids from gastrointestinal tract of the white rat: comparitive studies of the metabolism of amino acids."

Wilson, R.H. (1930).
J. biol. Chem. 87, 175. "The rate of absorption of cystine from the gastrointestinal tract of the white rat."

Wilson, R.H. (1932).
J. biol. Chem. 97, 497. "The effect of phlorhizin on the rate of absorption from the gastro-intestinal tract of the white rat."

Wood, H.O. (1944).
J. Anat. Lond. 78. "The surface area of the intestinal mucosa in the rat and in the cat."

Zunz, E. (1908).
Bull. Acad. Méd. Belg. 4s. 22, 588. "Contribution à l'étude de la digestion et de la résorption des proteines dans l'estomac et dans l'intestin grêle chez le chien."

---oOo---